

320

Bulletin of the

C. L. R. I. Madras

May 1959



52
m97, dm 211m NS4

NS9.5-10
136622

Bulletin of The Central Leather Research Institute

Vol. V

MAY, 1959

No. 10

CONTENTS

Studies on the Reactive Groups of Collagen : III. Methylation and Guanidination — K. Thomas Joseph & S. M. Bose ..	399
Research Bears Fruit : Practical Demonstration No. 4. (Third Series) Manufacture of Kattai and Bunwar Leathers — T. S. Krishnan, K. Rajabathar & K. T. Sarkar ..	412
Technical Seminar : Studies in Chrome Retannage — S. P. Singaram ..	422
Classified Abstracts ..	424
Notes & News ..	438
C. L. R. I. News ..	446

Editorial Board :

Dr. Y. NAYUDAMMA (*Editor*)

Dr. V. S. PADMANABHAN (*Managing 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 Managing Editor, C. L. R. I., Madras-20.

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



His Excellency Mr. Trampeyzski, Minister for Foreign Trade,
Poland, going round the Tannery of the C. L. R. I.

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

SCALE OF CHARGES FOR ADVERTISEMENTS

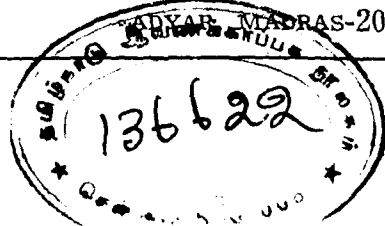
Screen 125

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

*Communications should be addressed to The Managing Editor,
Editorial Board.*

CENTRAL LEATHER RESEARCH INSTITUTE,

ADYAR, MADRAS-20.



**STUDIES ON THE REACTIVE GROUPS OF COLLAGEN:
III. METHYLATION AND GUANIDINATION ***

BY

K. THOMAS JOSEPH & S. M. BOSE
Central Leather Research Institute, Madras

Received on September 14, 1959.

SUMMARY

Collagen has been esterified with a number of reagents. Full esterification of collagen was achieved with methanolic HCl, methanol and thionyl chloride and methanol and ethyl chloroformate. A phenomenon accompanying the esterification of collagen with methanol and ethyl chloroformate was an increase in the amino nitrogen content. This has been traced to N-O-acyl migration in serine and threonine residues.

In a previous communication,¹ methods were described for the total and selective acylation of collagen and also for the destruction of the guanidino groups of collagen. Blocking or modification of the carboxyl groups of collagen is very essential for determining the role of this functional group in some of the tanning reactions. A number of the reagents which react with carboxyl groups of proteins, e.g., diazomethane, dimethylsulphate, methanol etc., are not specific to carboxyl groups^{2,3}. Herzig and Landsteiner⁴ studied the methylation of wool with diazomethane and after 18 treatments with this reagent, obtained wool with a methyl content of 5.89 and 6.28% of CH₃ linked to nitrogen. Edlbacher⁵ methylated a number of protamines with methyl sulphate and NaOH and he found besides the esterification of the carboxyl groups, some N-methylation also taking place. Felix and Rauch⁶ and Felix and Reindal⁷ prepared methyl esters of proteins by the action of dry HCl on their suspen-

* A paper submitted at the 46th Session of the Indian Science Congress, held in Jan. 1959.

sions in methyl alcohol. They found that the product could be further methylated by either methyl sulphate at pH 8 or 9 or by diazomethane. These more fully methylated proteins did not give the Pauly diazo reaction for the free imidazole ring or the Millon reaction for tyrosine, which showed that the imidazole ring of histidine and the phenolic group of tyrosine also reacted in the subsequent methylation.

Blackburn, Carter and Philips⁸ studied the methylation of wool with methyl sulphate and methyl halide and concluded that the main reaction was esterification of the carboxyl groups. Bowes and Kenten⁹ methylated collagen with methyl bromide and also with methyl sulphate in the presence of various buffers. The methoxyl content of the different collagens treated with methyl sulphate varied from 0.94 to 1.57% (as methyl), the maximum methoxyl content being attained in 14 methylations. Some of these conditions caused extensive breakdown of the protein structure. In fact the use of dimethyl sulphate treated collagen as a substrate for comparative tanning experiments has been strongly criticised.¹⁰ Burton, Danby and Sykes,¹¹ however, methylated collagen by alcoholic dimethyl sulphate in the presence of sodium borate and observed no sign of degradation or darkening of the product. The methanol and hydrochloric acid method¹² was found successful in producing a high degree of esterification but some degradation has been reported¹³ recently by this method.

Noller and Dutton¹⁴ have shown that trialkyl phosphates may be used to alkylate phenols and Billman *et al.*¹⁵ have applied them successfully to the alkylation of amines. Toy¹⁶ methylated aliphatic alcohols by using trimethyl phosphate. The commercial availability of trialkyl phosphates has created considerable interest regarding their use as alkylating agents but so far this reagent has not been tried for the methylation of proteins.

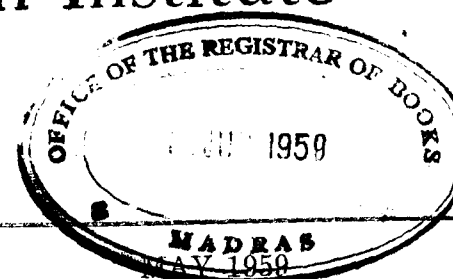
Freudenberg and Jakob¹⁷ have shown that 2, 3, 6 trimethyl glucose in methyl alcohol could be converted into

the Me-glucoside even at 20° in the presence of a little acetyl chloride which was superior to HCl as catalyst. They also found that certain acid halides have the same advantage over HCl in the esterification of organic acids. The acid chlorides successfully employed by them were acetyl chloride, ethyl chloroformate, thionyl chloride and stearoyl chloride. In all the cases methylation took place readily at 20°.

Brenner and Huber¹⁸ reported that alcohol and thionyl chloride gave good yields of amino acid esters while leaving the amino groups intact. Utilising the basic principle of this method Bello¹³ methylated gelatin specifically at the carboxyl groups. He found that the esterification of gelatin was largely complete in three hours and that the degradation was much less than that in the alcohol and HCl method. In the present case, a comparative study of the methylation of collagen has been made using different reagents namely dimethyl sulphate, trimethyl phosphate, methyl iodide, diazomethane, methanol and HCl, methanol and thionyl chloride and methanol and ethyl chloroformate and the degree of blocking or inactivation of the reactive groups of collagen has been quantitatively estimated in each case.

The conversion of amino groups of peptides and proteins to guanidino groups has received attention in recent years. It was first observed by Greenstein¹⁹ that O-methyl isourea reacts with lysine to form homoarginine. O-methyl isourea was first applied in the case of proteins, by Hughes, Saroff and Carney²⁰ for the guanidination of serum albumin and they found that the majority of the ϵ -amino groups could be converted to the guanidino groups. Roche *et al.*²¹ reported on the reaction of O-methyl isourea with several proteins including bovine serum albumin, casein and ovalbumin and they found that the reagent reacted only with the ϵ -amino groups of the proteins. Although several other biologically active substances have been guanidinated, no report has yet appeared on the guanidination of a fibrous protein like col-

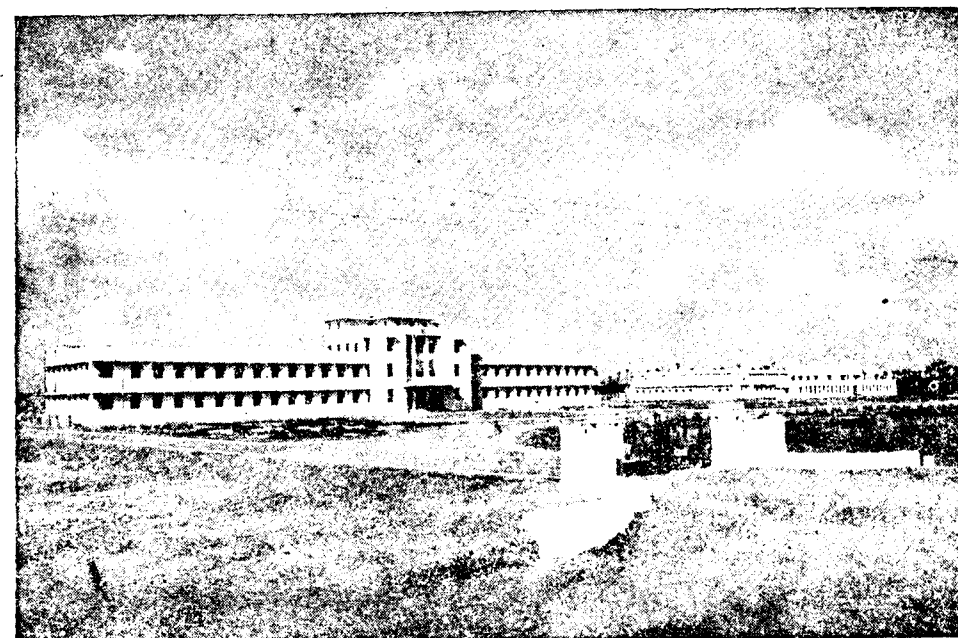
Bulletin of
The Central Leather
Research Institute
Madras



VOL. V

No. 10

340



COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH
INDIA

sions in methyl alcohol. They found that the product could be further methylated by either methyl sulphate at pH 8 or 9 or by diazomethane. These more fully methylated proteins did not give the Pauly diazo reaction for the free imidazole ring or the Millon reaction for tyrosine, which showed that the imidazole ring of histidine and the phenolic group of tyrosine also reacted in the subsequent methylation.

Blackburn, Carter and Philips⁸ studied the methylation of wool with methyl sulphate and methyl halide and concluded that the main reaction was esterification of the carboxyl groups. Bowes and Kenten⁹ methylated collagen with methyl bromide and also with methyl sulphate in the presence of various buffers. The methoxyl content of the different collagens treated with methyl sulphate varied from 0.94 to 1.57% (as methyl), the maximum methoxyl content being attained in 14 methylations. Some of these conditions caused extensive breakdown of the protein structure. In fact the use of dimethyl sulphate treated collagen as a substrate for comparative tanning experiments has been strongly criticised.¹⁰ Burton, Danby and Sykes,¹¹ however, methylated collagen by alcoholic dimethyl sulphate in the presence of sodium borate and observed no sign of degradation or darkening of the product. The methanol and hydrochloric acid method¹² was found successful in producing a high degree of esterification but some degradation has been reported¹³ recently by this method.

Noller and Dutton¹⁴ have shown that trialkyl phosphates may be used to alkylate phenols and Billman *et al.*¹⁵ have applied them successfully to the alkylation of amines. Toy¹⁶ methylated aliphatic alcohols by using trimethyl phosphate. The commercial availability of trialkyl phosphates has created considerable interest regarding their use as alkylating agents but so far this reagent has not been tried for the methylation of proteins.

Freudenberg and Jakob¹⁷ have shown that 2, 3, 6 trimethyl glucose in methyl alcohol could be converted into

the Me-glucoside even at 20° in the presence of a little acetyl chloride which was superior to HCl as catalyst. They also found that certain acid halides have the same advantage over HCl in the esterification of organic acids. The acid chlorides successfully employed by them were acetyl chloride, ethyl chloroformate, thionyl chloride and stearoyl chloride. In all the cases methylation took place readily at 20°.

Brenner and Huber¹⁸ reported that alcohol and thionyl chloride gave good yields of amino acid esters while leaving the amino groups intact. Utilising the basic principle of this method Bello¹³ methylated gelatin specifically at the carboxyl groups. He found that the esterification of gelatin was largely complete in three hours and that the degradation was much less than that in the alcohol and HCl method. In the present case, a comparative study of the methylation of collagen has been made using different reagents namely dimethyl sulphate, trimethyl phosphate, methyl iodide, diazomethane, methanol and HCl, methanol and thionyl chloride and methanol and ethyl chloroformate and the degree of blocking or inactivation of the reactive groups of collagen has been quantitatively estimated in each case.

The conversion of amino groups of peptides and proteins to guanidino groups has received attention in recent years. It was first observed by Greenstein¹⁹ that O-methyl isourea reacts with lysine to form homoarginine. O-methyl isourea was first applied in the case of proteins, by Hughes, Saroff and Carney²⁰ for the guanidination of serum albumin and they found that the majority of the ε-amino groups could be converted to the guanidino groups. Roche *et al.*²¹ reported on the reaction of O-methyl isourea with several proteins including bovine serum albumin, casein and ovalbumin and they found that the reagent reacted only with the ε-amino groups of the proteins. Although several other biologically active substances have been guanidinated, no report has yet appeared on the guanidination of a fibrous protein like col-

lagen. This reaction is comparatively simple and, unlike the destruction of the guanidino groups other protein groups are not affected. Hence it was thought that the guanidination of collagen will be beneficial for studying the fixation of certain tanning agents by collagen. An attempt was therefore made to guanidinate collagen by the use of O-methyl isourea sulphate and the degree of modification was suitably followed.

Experimental

Collagen was prepared from the butt-portion of a fresh buffalo hide as described in a previous communication.²²

I. Methylation

(a) *With methanol and thionyl chloride*: The conditions adopted by Bello¹³ were followed. After the reaction, the product was neutralised to pH 6, washed, dehydrated in acetone and air dried. The catalytic effect of small amounts of pyridine on the reaction between thionyl chloride and a carboxylic acid is well known.²³ It was, therefore, also attempted to methylate collagen with methanol and thionyl chloride in the presence of pyridine but the results were almost identical with the previous method.

(b) *Esterification with dimethyl sulphate*: The method of Burton, Danby and Sykes¹¹ was followed. After reaction, the product which was in a highly swollen condition was placed in 10% salt solution overnight and then washed with distilled water and dehydrated with acetone.

(c) *Esterification with methanol and acid catalysis*: The method developed by Fraenkel-Conrat and Olcott¹² as adopted by Burton, Danby and Sykes¹¹ was followed.

(d) *Esterification with diazomethane*: The method suggested by Chibnall *et al.*^{24, 25} was adopted.

(e) *Methylation with trimethyl phosphate*: None of the conditions adopted by the previous workers^{14, 15, 16} for the alkylation of phenols, amines and alcohols could be followed in the case of proteins as the protein molecule was

prone to undergo degradation under these conditions. So in the present study comparatively mild conditions were adopted for the methylation of collagen. The method adopted was similar to that of Burton *et al.*¹¹ for the methylation of collagen with methyl sulphate.

Collagen (10 gms) was treated with 50 ml. of 10% alcoholic trimethyl phosphate and 200 ml. of 2% sodium borate and shaken in a shaking machine. The reaction medium was changed after every one hour until 10 treatments were given after which the product was washed and dehydrated in acetone.

(f) *Methylation with methyl iodide*: The method of Bowes and Kenten⁹ was followed with the substitution of methyl bromide by methyl iodide.

(g) *Esterification with methanol and ethyl chloroformate*: Collagen (5g) was suspended in 200 ml. of absolute methanol and 4 cc. of freshly distilled ethyl chloroformate was added and the contents shaken intermittently for 3 days at the end of which the mixture was filtered and the collagen washed repeatedly with methyl alcohol and twice with ether and dried in a vacuum desiccator. In another set of experiments, the volume of ethyl chloroformate was increased and the reaction time also was increased from three to five days.

Estimation of methoxyl groups: The total methoxyl groups in methylated collagen were estimated by the method of Belcher, Fildes, and Nutten.²⁶ As collagen contains methionine which yields MeI on treatment with HI the values reported were corrected for the methionine present, by having a blank with unmethylated collagen. The free amino nitrogen was determined by the modified method of Doherty and Ogg.²⁷ The amide nitrogen was estimated by the method of Burton, Danby and Sykes.¹¹ The amount of N-alkyl substitution was calculated from the free amino group nitrogen values. The reduction in the free amino

group was assumed to be due to the formation of N-alkyl derivatives. The results obtained with the analysis of all the methylated samples are given in Table I.

TABLE I
Analysis of methylated collagen. Values are expressed as milli moles per gm. of collagen

Treatment	Methoxyl groups introduced	Free amino-nitrogen
None	.. 0.05	0.37
Diazomethane, acidic 85% ethanol	.. 0.50	0.27
Trimethyl phosphate (10 treatments)	.. 0.74	0.05
Dimethyl sulphate (10 treatments)	.. 0.71	0.04
Methanol/HCl.	.. 0.82	0.30
Methyl iodide (treated for 14 days)	.. 0.21	0.30
Methanol and thionyl chloride	.. 0.78	0.36
Methanol and ethyl chloroformate:		
(1) Treated for 3 days	.. 0.84	0.43
(2) Treated for 5 days with (6 cc. of ethyl chloroformate)	.. 0.86	0.54

From the above table, it can be seen that when collagen is methylated with methanol and ethyl chloroformate there is an increase in the free amino groups. Chibnall, Mangan and Rees²⁵ have also recently reported that when insulin and β -lactoglobulin are methylated in methanolic HCl, there is an increase in the free amino nitrogen content. They discussed the possibility that this might be due to an N-O-acyl shift at serine and threonine residues to give the corresponding O-peptidyl ester and free amino group. Elliot²⁸ had shown that such change could take place in silk fibroin under the influence of strong mineral acid. In order to ascertain whether such a phenomenon has taken place in the present case, the following experiments were carried out: (1) the free amino nitrogen was redetermined after keeping the methylated collagen in a buffer solution at pH 9 for several hours. Elliot²⁸ has shown that under such conditions the O-peptidyl structure would be reconverted to

the normal N-peptidyl form by the action of the alkali; (2) The methylated collagen was coupled with dinitro-fluorobenzene before and after treatment with the pH 9 borate buffer and the amino acids having the free amino groups were identified. Since coupling with DNFB under the usual conditions will bring about the reverse change, the reaction was carried out in *M-acetate* buffer of pH 5.²⁸ The DNP amino acids were identified by the paper chromatographic technique described in an earlier communication.²²

The results obtained with these experiments are presented in Table II.

TABLE II
Free amino groups of collagen methylated by methanol and ethyl chloroformate. Values are expressed as milli moles per gm. of collagen

Substance	Free amino groups	DNP-amino acids identified				
		Aspartic acid	Serine	Threonine	Alanine	Glycine
Collagen methylated with methanol and ethyl-chloroformate (treated for five days)	.. 0.54	0.014	0.052	0.048	trace.	0.013
Do. (after treating with borate buffer of pH 9 for 5 hours)	.. 0.40	0.013	0.012	0.008	0.007	0.014

II. Guanidination

In the present study collagen was guanidinated by O-methyl isourea neutral sulphate which was synthesised by the method of Bello.²⁹

For guanidination 10 gms. of collagen were suspended in 150 ml. of 0.5 M solution of O-methyl isourea sulphate in water adjusted to pH 10.5 with KOH solution. The reaction was allowed to take place at 4°C for five days. The container was kept closed during the reaction as exposure to air decreased the pH and the rate of reaction. The pH of the solution was occasionally checked and maintained at 10.5 by the addition of KOH. After the reaction was over, the swollen collagen was neutralised with dilute acetic acid and washed with water for 2 hours. The extent of guanidination was followed by estimating the amounts of arginine and homoarginine in the acid hydrolysate of the treated collagen by the method of MacPherson³⁰ and also by determining the amino nitrogen by the modified method of Doherty and Ogg.²⁷

The results obtained on the analysis of guanidinated collagen are given in Table III.

TABLE III
Characterisation of guanidinated collagen
Values are expressed as milli moles per gm. of collagen

Method	Collagen	Guanidinated collagen	Percent amino groups reacted
Free amino groups	0.37	0.14	62.5
Arginine and homo-arginine	0.54	0.79	65.0

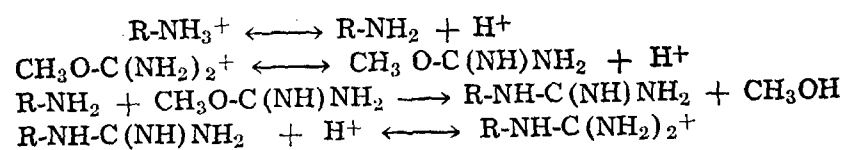
Discussion

When collagen is esterified with diazomethane or methyl iodide, only a small percentage of the carboxyl groups are

methylated. Moreover, the reagents are not selective to carboxyl groups and the rate of the reaction is also found to be extremely slow. Greake and Nierenstein³¹ found that for the complete methylation of casein by diazomethane about four months were required.

From Table I it can be seen that dimethyl sulphate and trimethyl phosphate are more efficient methylating agents than diazomethane or methyl iodide but in these two cases also the reaction is not specific to the carboxyl groups and extensive N-methylation takes place as is evident from the free amino group determinations. The methanol and hydrochloric acid method was successful in producing a high degree of esterification, but some degradation has been reported recently during the long reaction time. Chibnall, Mangan and Rees²⁵ have recently reported the esterification of insulin and β -lactoglobulin with diazomethane and methanolic HCl. They found that neither reagent was entirely satisfactory. Diazomethane did not give full esterification while hydrolytic loss of amide nitrogen was observed in the case of methanolic HCl. In the present study, however, no appreciable loss of amide nitrogen could be observed in the case of collagen methylated with methanolic HCl. Fraenkel-Conrat and Olcott¹² could not observe any loss of amide N when proteins were esterified with methanol and HCl.

In the case of esterification with methanol and thionyl chloride and also by methanol and ethyl chloroformate, a large amount of methoxyl groups were introduced. The amino groups were also not methylated as indicated by the amino nitrogen determination. A phenomenon accompanying the esterification of collagen by methanol and ethyl chloroformate is an increase in the free amino nitrogen. Chibnall *et al.*²⁵ have discussed the possibility of an N-O-acyl shift at serine and threonine residues to give the corresponding O-peptidyl ester and free amino group when certain proteins like insulin and β -lactoglobulin were methy-



Thus in the reaction of O-methyl isourea with proteins, no drastic change in the charge pattern is produced and no chemical grouping is introduced which is foreign to biological systems. The reaction is also comparatively simple and unlike with certain other reactions, other protein groups are not affected.

Acknowledgment

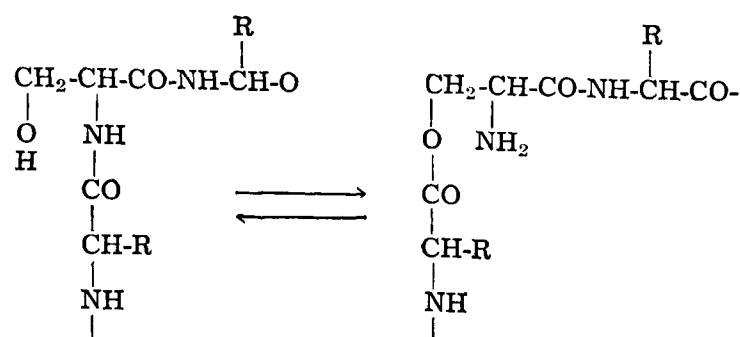
Our thanks are due to Dr. Y. Nayudamma, Director, Central Leather Research Institute, for his keen interest in this work and for permission to publish these results.

REFERENCES

1. Bose, and Joseph, *Arch. Biochem. and Biophys.*, **74**, 46 (1958).
2. Herriot. *Adv. in Prot. Chem.* **3**, 169 (1947).
3. Olcott and Fraenkel-Conrat., *Chem. Rev.*, **41**, 151 (1947).
4. Herzig and Zandsteiner, *Mh. Chem.*, **39**, 369 (1918).
5. Edlbacher, *Z. Physiol. Chem.*, **107**, 58; **108**, 287 (1919).
6. Felix and Rauch, *Z. Physiol. Chem.*, **200**, 27 (1931).
7. Felix and Reindal, *Z. Physiol. Chem.* **205**, 11 (1932).
8. Blackburn, Carter and Philips, *Biochem. J.* **35**, 627 (1941).
9. Bowes and Kenten, *Biochem. J.*, **44**, 142 (1949).
10. Kremen and Lollar, *J.A.L.C.A.*, **46**, 34 (1951).
11. Burton, Danby and Sykes, *J.S.L.T.C.*, **37**, 219 (1953).
12. Fraenkel-Conrat and Olcott, *J. Biol. Chem.*, **181**, 259 (1945).
13. Bello, *Biochem. Biophys. Acta* **20**, 426 (1956).
14. Noller and Dutton, *J. Am. Chem. Soc.*, **55**, 424 (1933).
15. Billman et al, *J. Am. Chem. Soc.*, **64**, 2977 (1942).
16. Toy, *J. Am. Chem. Soc.*, **86**, 499 (1944).
17. Freudenberg and Jakob, *Ber.*, **74B**, 1001 (1941).
18. Brenner and Huber, *Helv. Chim. Acta* **38**, 1109 (1953).
19. Greenstein, *J. Biol. Chem.*, **109**, 529, 541 (1935).
20. Hughes, Saroff and Carney, *J. Am. Chem. Soc.*, **71**, 2476 (1949).

21. Roche, Mourgue and Baret, *Bull. Soc. Chim. Biol.*, **36**, 85 (1954).
22. Joseph and Bose, *Bull. C.L.R.I.*, **4**, 343 (1958).
23. Human and Mills, *Nature* **158**, 877 (1946).
24. Chibnall and Rees, *Biochem. J. Proc.*, **48**, XLVII (1951).
25. Chibnall, Mangan and Rees, *Biochem. J.*, **68**, 114 (1958).
26. Belcher, Fildes and Nutten, *Anal. Chim. Acta* **13**, 16 (1955).
27. Doherty and Ogg., *Ind. Eng. Chem. Anal. Ed.* **15**, 751 (1943).
28. Elliot, *Biochem. J.*, **50**, 542 (1952).
29. Bello, *Biochim. Biophys. Acta* **18**, 448 (1955).
30. MacPherson, *Biochem. J.* **40**, 472 (1946).
31. Greake and Nierensterin, *Biochem. J.*, **8**, 287 (1914).
32. Chervenka and Wilcox, *J. Biol. Chem.* **222**, 635 (1956).
33. Geschwind and Li., *Biochim. Biophys. Acta.* **25**, 171 (1957).

lated with methanolic HCl. The reaction which is reversible can be represented as follows:



In the present study no increase in amino nitrogen could be detected in collagen treated with methanolic HCl but the increase in free amino groups was quite appreciable in the case of methylation with methanol and ethyl chloroformate. As shown in Table II the amino nitrogen in the treated protein was considerably diminished after the material had been kept in borate buffer of pH 9 for several hours. This shows that the N-O-acyl shift has taken place in this particular reaction. The data presented in table II show that both the serine and threonine residues are concerned in the reaction as both DNP-serine and DNP-threonine were detected in the treated collagen after coupling with DNFB in pH 5 acetate buffer. The concentration of DNP-serine and DNP-threonine were considerably decreased when the methylated collagen was kept in borate buffer. Other DNP-amino acids detected were glycine, alanine and aspartic acid. It may be mentioned in this connection that aspartic acid, glycine, and serine were detected in traces as the N-terminal amino acids of collagen in a previous study.²² But the amount of DNP serine detected in the methylated collagen was considerably in excess of the reported N-terminal serine value for collagen. These results, therefore, show that the N-O-acyl shift has taken place both at the serine and threonine residues. Since there is no appreciable increase in the free amino groups when collagen is methy-

lated by the other methods, it appears that it is the catalytic effect of ethyl chloroformate which promotes the acyl shift, and not the esterification of the carboxyl groups.

In the case of guanidination of collagen by O-methyl isourea, it has been found (Table III) that about 65% of the ϵ -amino groups could be converted to the guanidino groups. Collagen behaved differently from certain other proteins with respect to the extent of guanidination. Chervenka and Wilcox³² found that almost the whole of ϵ -amino groups in chymotrypsinogen could be converted to the guanidino groups by reaction with O-methyl isourea. Geschwind and Li³³ reported that the ϵ -amino groups of lactogenic hormone and lysozyme could be fully guanidinated while those of growth hormone were only 90-95% guanidinated under similar conditions. However, difficulties in complete guanidination were experienced by Roche *et al.*²¹ in the case of thyroglobulin. Geschwind and Li³³ also attempted to guanidinate a number of other proteins and they found that under none of the conditions employed could either insulin or α -corticotropin be completely guanidinated. In α -corticotropin only three out of four lysine residues were converted to homoarginine. In view of these reports it is, therefore, not surprising that in collagen which is an insoluble fibrous protein only about 65% of the ϵ -amino groups could be guanidinated under the conditions of the experiment employed.

Hughes *et al.*²⁰ have reported that the rate of reaction of methyl isourea with albumin increases with pH indicating that the reaction involves the unionized form of one or probably both of the reactants. Thus for quantitative reaction, the maximum pH at which the protein is stable will be desirable and hence with most of the proteins the reaction has been carried out at pH 10.5. At this pH the more basic guanidino group formed by the reaction of O-methyl isourea with the less basic amino group will be almost completely in the charged guanidinium form.

RESEARCH BEARS FRUIT

Practical Demonstration No. 4

(Third Series)

Manufacture of Kattai and Bunwar Leathers *

BY

T. S. KRISHNAN, K. RAJABATHAR & K. T. SARKAR

Manufacture of Kattai leather from raw buff calfskins

Raw materials : Wet salted buff calfskins weighing upto 10 lbs. per piece.

Soaking : The skins are weighed after removing the excess of salt and this weight is taken as the wet salted (W/S) weight. They are soaked in plain water for 1-3 hours after which three or more washes with plain water are given. The well soaked skins are drained and if necessary the soaked weight is determined.

Liming and unhairing : 10-15% slaked lime and $\frac{1}{2}$ % sodium sulphide on the W/S weight are added to once used lime liquor i.e., the liquor from which the skins of the previous lots have been taken for fleshing. The sodium sulphide is dissolved in hot water and added. After stirring up the liquor well, the skins are put in the liquor and handled twice. The same evening another handling is given. On the 2nd day, the hides are removed and the lime liquor is strengthened by the addition of another $\frac{1}{2}$ % sodium sulphide (previously dissolved in hot water). The liquor is stirred well and the goods are put back and handled twice, once in the morning and once in the evening. On the 3rd

day another handling is given and on the 4th or 5th day the goods are unhaired and are ready for reliming.

Reliming : The unhaired skins are put in a bath consisting of sufficient fresh water, 10% slaked lime and $\frac{1}{4}$ % caustic soda on the W/S weight. The hides are handled in this, twice daily, once in the morning and once in the evening and they remain here for 3 days. On the 4th day the goods are fleshed, weighed, washed in water for 30 minutes, after which they are scudded and put in plain water overnight.

The washing with water may be done in a drum or paddle or by trampling in a pit.

Deliming : Next day, the skins are trampled, fleshed, washed, scudded, washed and delimed fully (cut edge should give no colouration when tested with phenolphthalein) with 1-2% boric acid and sufficient water. The deliming may be done in a pit or drum or paddle.

The delimed skins are scudded well, washed, drained and are ready for tanning.

Liquor process of pit tannage : In this, tan liquor is prepared separately and skins are soaked in tan liquor of increasing strength. The tan liquor is prepared by leaching 3 parts of wattle bark and one part of crushed myrobalam nuts. Instead of wattle bark, $1\frac{1}{2}$ parts of wattle extract may be used with myrobalam nuts.

The drained skins are suspended in a weak mellow liquor of 7-10° BK. Next day, the pack is transferred to 10-12° BK and the skins are rocked several times for the first two days so that there may be uniform colouration and penetration.

3rd day:—Beaming on flesh side and suspending or laying flat in 15° BK liquor.

4th day:—Handling.

* Process of the practical demonstration begun in May 1959.

5th day:—Piling and putting in 20° BK liquor by laying flat.
 6th day:—Handling.
 7th day:—Beaming and putting in 25° BK liquor by laying flat.
 8th day:—Handling.
 9th day:—Piling and putting in 30° BK liquor by laying flat.
 10th day:—Handling.
 11th day:—Beaming and putting in 35° BK liquor by laying flat.
 12th day:—Handling.
 13th day:—Piling and putting in 40° BK liquor by laying flat.
 14th day:—Handling.
 15th day:—Beaming if necessary and putting in 45° BK liquor by laying flat.
 16th day:—Handling.
 17th day:—Piling and putting in 50° BK liquor by laying flat.
 18th day:—Handling.
 19th day:—Piling and putting in 55° BK liquor by laying flat.
 20th day:—Handling.

The tanned skins are piled for a day or two keeping them grain to grain and covering well.

They are then weighed (sammed weight) and scoured well on both sides.

Washing and fat liquoring : The tanned skins are washed in a drum with plain water for 5-10 minutes and are then fat liquored for 1 hour with 150% water at 45°C and 5-6% fat liquor on the sammed weight. (3 parts of Sulfonated oil and 1 part of free oil).

After 1 hour drumming, ¼% formic acid, diluted with water is added in slow feeds and drumming is continued for

another 20 minutes. The fat liquored leathers are piled over a horse and covered.

Next day the leathers are hung up in a dark drying room. When semi-dry they are set once and dried completely. After drying they are kept in the crust form.

The crust leathers are dipped in water and piled overnight. Next morning they are dry drummed for 1-2 hours and in the sammed condition, splitting and shaving are done to the required thickness. The shaved stock is then weighed. A plain wash with water is given for 20 minutes after which they are bleached in a drum with 200% water and ½-¾% oxalic acid for 20 minutes followed by another plain water wash. The washed goods are taken out and drained.

Retanning and currying : The goods are retanned with *Dhawa* (*Anogeissus latifolia*) or myrobalam liquor of 15° BK for 1-2 hours, after which they are rinsed and sammed. The skins are set on the flesh side and the grain side is oiled with a little of linseed (raw) or neatsfoot and mineral oil. They are then hooked for semi-drying and in the right condition (the dried portions are wetted with water) the hides are put on the setting table with flesh side up and dubbin is then applied on the flesh side uniformly, taking care to see that the grain does not come into contact with dubbin.

After applying dubbin on the flesh side, the leathers are put with grain side up and the grain side is set with a slicker. Setting should be done with great care and strength such that the folds marks, etc., especially in the neck and shank portions are removed. They are then hung up and after semi-drying they are again set and hung up for complete drying. Just before drying they are finally set with a glass slicker.

Preparation of Dubbin: Tallow is first melted and then the oil is added slowly with constant stirring until it becomes semi solid.

Composition of dubbin varies according to the season and place. Generally in summer, more tallow is used and in winter less tallow. The semi solid dubbin is converted to a smooth pasty mass by beating it with hand. If necessary a little water can be used.

Composition of Dubbin:

- 3 parts of tallow.
- 1 part of fish oil.
- 1 part of Linseed oil (raw unboiled).

The dried and curried leathers are kept piled for 2-3 days.

Finishing operations: The curried leathers are then sorted according to grain defects, patchiness, etc. Those which are free from patchiness and numerous grain defects are taken for light shades and the rest for dark shades.

The leathers are staked lightly, trimmed and the grain side is buffed with 180 grit paper followed by buffing with 240 paper. Sorting should be done for proper buffing. They are then brushed to remove the buffing dust from the grain surface.

Stain Coat :—

For colours.

- 1 part of acid dye (suitable)
- 1 part of acetic acid
- 100 parts of water.

The grain side is padded or brushed liberally with the stain coat and dried.

Filling coat: 2½% solution of linseed mucilage is prepared and filtered. This is applied on the grain side liberally and dried.

Stain coat and filling coat may be mixed and given in one coat.

The dried leathers are then smoothplated under low pressure.

(a) Pigment Season :

- 12-20 ozs. of pigment paste (adjusted to proper shade).
- 4- 7 gms. of acid dye.
- 20 ozs. of 10% casein solution.
- 20 ozs. of 10% linseed mucilage solution.
- 10 ozs. of 10% gelatin solution.
- 4 ozs. of binder.
- 1½ ozs. of T.R.O.
- 1½ ozs. of glycerine.
- 1 oz. of ammonia.
- ½ oz. of nitrobenzene.
- 1 gallon of water.

The dried leathers are then given two pad coats followed by a spray coat.

Before giving the second pad coat, 2 ozs. of formaldehyde diluted well with water is added to 1 gallon of above season.

The sprayed leathers are assorted for proper coverage and are then staked lightly.

Top Coat :

- 4 ozs. of 10% casein solution.
- 4 ozs. of egg albumen or Neeran glazing finish A. (I.C.I.).
- 20 ozs. of water.

The staked leathers are given a spray coat of top season and dried.

Fixing Coat :

- 5% casein solution—1 part.
- 10% formaldehyde—1 part.

Washing and fat liquoring : The goods are washed in a drum for 5-10 minutes after which they are fat liquored with

5% sulfonated fish oil,
1½% castor oil,
0.05% paranitrophenol,
200% water at 45°C.

for 1 hour after which ¼ to ½% formic acid diluted well with water is added in slow feeds. Drumming is continued for another 20 minutes and the leathers are taken out and piled.

Next day semi-drying, setting, oiling off, currying and finishing are done as described before for Kattai leathers.

Where drumming facilities are not available, the retanning may be done in a pit by keeping the skins in a liquor of 30° BK for 3 days and in 40° BK for another 3-4 days.

Bunwar leather from E. I. buff hides

Raw materials : E. I. buff hides.

The hides are soaked in water to which 1% wetting agent (based on soaked and sammed weight) is added. Overnight soaking is preferable and the goods are drummed in the same bath for another 20-30 minutes. A wash with plain water is given after which the goods are taken out and drained by piling. Shaving is done if necessary. The goods are retanned with 40°BK liquor in a drum for 6-8 hours and they are left in the liquor overnight. The proportion of tanstuff may be as in the case of Kattai leather from E. I. tanned buff calf.

Next morning the goods are taken out and are retanned with 60° BK liquor for 6-8 hours, and they are left in the liquor overnight.

On the 3rd day, they are taken out and piled for a day. The goods are then scoured, washed in a drum for

5-10 minutes and fat liquored with 4-6% fat liquor composed of sulphonated fish oil and untreated fish or castor oil. The fat liquored goods are semidried and during drying 3 or 4 settings are given and they are then completely dried.

If drumming facilities are not available, the goods after soaking and draining are put in a pit containing 30° BK for 3 days.

They are then put in 45° BK for another 3 days followed by 60° BK for another 3 days.

The dried goods are finished as described under Bunwar leathers from liquor tanned hides.

The leathers are sprayed by fixing coat and dried well. The well dried leathers are then glazed and assorted.

(b) *Finishing with Synthetic resin :*

Pigment Season :

- 1 part of pigment paste (adjusted to proper shade)
- $\frac{3}{4}$ part of synthetic resin emulsion.
- $\frac{1}{2}$ part of casein 10% solution.
- $\frac{1}{4}$ part of 10% Carnauba wax emulsion.
- $\frac{1}{16}$ part of ammonia.
- $2\frac{1}{2}$ - $3\frac{1}{2}$ parts of water.

A pad coat of the above season followed by two spray coats to cover are given. The leathers can be cold smooth plated under low pressure and then two spray coats can be given.

They are then sprayed with the fixing coat and dried well.

The leathers are then hot plated at 150°F and at 150 Kg/sq. cm. pressure.

Bunwar leather from raw buff hides

Raw materials : W/S buff hides weighing upto 28 lbs.

Soaking, liming, reliming are the same as those described for Kattai (buff calf skins) leathers. The deliming is done two-thirds and the tannage is the same as for Kattai except that the tanning is carried out upto 65° BK.

The tanned leathers are piled for a day or two, then scoured well, washed for 5-10 minutes and fat-liquored with 3-5% fat liquor. They are then piled overnight and are then semidried. During drying two or three settings are given and the leathers are then completely dried.

The dried leather is lightly rolled after applying soap solution on the grain surface.

The leathers are then trimmed, buffed on the grain surface with 180 grit paper and then brushed to remove dust.

In case of Bunwar, staining is done if necessary and the rest of the operations like pigmenting, glazing, etc., are the same as for Kattai but no staking and flesh buffing are required for Bunwar leather.

Kattai leather from E. I. buff calf

Raw materials : E. I. tanned buff calfskins.

Preconditioning, splitting and shaving : The skins are dipped in water and piled overnight. Next day they are dry drummed for 1 to 2 hours and in the sammed state, they are split and shaved to required thickness. The shaved skins are then weighed and are soaked in a drum with 300-400% water to which 1% wetting agent (Teepol or Lissapol) may be added (calculated on the shaved weight). The goods are drummed in the same bath for 15 minutes after which another wash with plain water for 20 minutes is given.

Bleaching : The soaked goods are bleached in a drum for 20 minutes with 200% water and $\frac{1}{2}$ - $\frac{3}{4}$ % oxalic acid after which a plain wash with water for 15 minutes is given. The bleached goods are taken out and drained by piling over a horse.

Retanning : The tan liquor is prepared from 1½ parts of mimosa extract and 1 part of crushed myrobalam nuts and 4% sodium bisulfite (on the weight of extract). Sufficient water is added to make the strength of tan liquor 40° BK.

The goods are drummed for 6-8 hours with 150% of 40° BK liquor.

The drum is run and stopped alternately for ½ hour intervals.

The goods are left immersed in the bath. Next day the goods are taken out, rinsed in water and piled.

TECHNICAL SEMINAR

Studies in Chrome Retannage*

BY

S. P. SINGARAM

Chrome retannage imparts fullness to the leather and makes the grain tight and compact. The overall thickness of the leathers also is increased making them suitable for use as uppers for army and police shoes, which is not the case with straight chrome tannage. Light retanning of civilian grade upper leathers upgrades the quality and increases the cutting value. The main disadvantage of chrome retannage is the grain crackiness of the retanned leather especially on ageing. The reasons for this are : (1) Use of vegetable retanning agent having higher amounts of non-tans, resins and mucilage and (2) displacement, on ageing, of the acidic groups of the retanned leather, and (3) insufficient and improper fat-liquoring. The present studies have been made with the aim of rectifying this defect, selecting suitable indigenous retanning materials and also working out optimum conditions of processing prior to retannage.

Tan and combining values, water solubles content of the retanned leather and effective increase in thickness on retanning have been studied with a number of indigenous vegetable retanning materials. Karada, Goran and Cutch are recommended as the most suitable. The optimum conditions for use of the vegetable tanning materials for retanning have been worked out.

* Condensed from the talk given on 17-4-1959.

Optimum pickling conditions have been worked out based on layerwise fixation of Cr_2O_3 . The use of Calgon in pickling also has been studied. In pre-chroming the three different ionic types of chrome liquors have been tried and it has been concluded that only cationic pre-chroming makes possible fixation of the increased amounts of vegetable tannins necessary for successful re-tannage. Smaller amounts of chrome have been found sufficient for accelerated vegetable tannin fixation and for obtaining higher shrinkage temperature. The inactivity and suppressing effect of the non-ionic and anionic chrome liquors in regard to the fixation of vegetable tannins can be advantageously used where such effects are needed. By using Calgon as neutralising agent, tannin fixation on the grain is retarded, thus producing the desired fullness and absence of grain crackiness.

CLASSIFIED ABSTRACTS

PROCESSES PRIOR TO TANNING

BRINE TREATMENT OF SHEEP SKINS. I. Leont'ev. *Myasnaya Ind. S.S.S.R.* 29 No. 1, 17-19 (1958); *Chem. Abst.* 52, 17769 (1958). Preservation of sheep skins in concentrated salt brines instead of by using dry salt is more efficient regarding the storage life of the skins, spoilage under exceptional conditions at 35-6° and 85-90 % relative humidity for 2 months, and the yield of the finished product, without affecting quality of the wool.

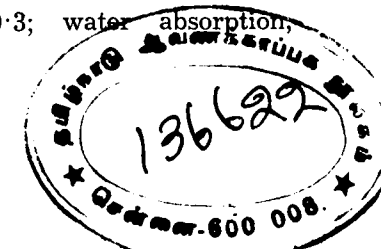
PRESERVATION OF HIDES IN BRINES OF HIGH SPECIFIC GRAVITY. A. Anfimov, V. Leites and N. Smol'ski. *Myasnaya Ind. S.S.S.R.* 29, No. 1, 15-17 (1958); *Chem. Abst.* 52, 17769 (1958). Cattle hides were cut into sides. Control sides were kept in saturated salt brine, sp. gr. 1.2 and experimental sides in the salt brine containing either 7.7 % (I) or 15.8 % (II) $(\text{NH}_4)_2\text{SO}_4$ plus Na_2SO_4 (sp. gr. 1.222-1.224 and 1.265-1.272 resp.) The brine treatment was made for 8, 12 and 16 hrs. followed by storage of the hides for 3 months. The treatment of hides in the experimental brines I and II dehydrated the hides more than the control brine, the effect being greater with increasing ammonium salts. The following chemical indexes were found on the hides brine-treated for 16 hrs. and then stored for 3 months (in the order of control, I and II): moisture 51.40-55.40, 48.67-50.54, and 45.24-47.76 (after brine treatment) and 44.89-46.62, 43.07-44.89 and 43.65-44.13% (after storage); NaCl 12.75-13.17 and 12.15-12.89 and 11.84-13.0 and 16.26-16.58, 15.12-16.06, and 14.93-15.27% respectively. The sides treated in I and II contained 3.08 and 6.61-7.36% $(\text{NH}_4)_2\text{SO}_4$ after 3 months storage. The treatment in II decreased the total bacterial count of the hides from 47.75-189 to 25.88-50.5 $\times 10^4$ respectively. The decrease affected proteolytic and halophilic bacteria and molds.

THE CONTROL OF WASHING AND DELIMING PELTS. Anton Blazej and Elena Markusovska (*Tech. Univ., Slovakia, Bratislava, Czech*). *Kožarství* 7, 357-9 (1957); *Chem. Abst.* 52, 17767 (1958). CaO is present in pelt partly as H_2O -soluble $\text{Ca}(\text{OH})_2$,

Ca salts of amino acids, and insolubly bound to COOH groups of proteins. After washing 8 hrs. the pelt still retains 29 % of the insoluble Ca. Control of Ca content by spotting with phenolphthalein is only qualitative. The colorimetric method of Stehlik and Podolsky (formation of a colored complex with pyrogallol-carboxylic acid) is limited to the range 0.2 to 1.0 mg. Ca in solution and preparation of permanent standards is impossible because of instability of the compound. Chelametric detn. of Ca with complexon III (I) (disodium salt of ethylene diaminetetraacetic acid) is proposed. Cut a 1 $\times$ 2-3 cm. strip from the butt, weigh to 0.01 g., hydrolyze by boiling with 30 ml NH_4Cl , and add 50 ml. H_2O , neutralize to methyl orange with 2N NaOH, and add 2 ml. 2 N NaOH and 10 ml. of buffer solution containing 2 g. NH_4Cl and 100 ml. concentrated NH_4OH per l. Add 0.3 g. of murexide and titrate with 0.01 M I (3.721 g. per l.) to the change from reddish orange to blue-violet. Hydrolyzed hide substance up to 1.5 g. N does not interfere. Limed steer pelt contained 2.80-2.85 % CaO (on dry basis), 2.01-2.12 % after washing, 1.61-1.64 % after deliming, 1.15-1.19 % after bating and 0.28-0.35 % after pickling. These differences are the max. found in determinations.

OTHER TANNAGES

DIHYDROXYBENZENES AS RAW MATERIALS FOR THE PRODUCTION OF SYNTANS IN CZECHOSLOVAKIA. Antonin Kala (*Leather & Allied Trades Research Inst., Gottwaldov, Czech*). *Věda a výzkum v průmyslu kožedělném* 3, 53-61 (1958); *Chem. Abst.* 52, 17770 (1958). The comparison of the dihydroxy benzene fraction of the middle oil fraction of soft coal tar is very variable. A commercial product "Difen" (I) contained pyrocatechol 26, 4-homopyrocatechol 22, 3-isopyrocatechol 8, and other higher phenols 15.5 %. The constitution and properties of syntans made from I are: Omega syntan contains tannin 41.6 nontannin 4.6 % and has combining values of 44.5 at 3 hrs. and 48.1 at 24 hrs. OM₂ has combining values of 50.5 and 53.6. A new syntan, P₂, made on a pilot-plant scale from technical pyrocatechol and HCHO, contains no SO_3H . It contains 69 % tannin and only 0.8 % nontannin and has combining values of 70.9 and 78.8. In contrast, K₂D a widely used syntan, contains only 35 % tannin, 6% nontannin, and has combining values of 53.3 and 62.1. Leather tanned with mixtures in which either P₂ or K₂D constituted 50% of the total tannin showed: degree of tannage, P₂ 88.7, K₂D 70.3; water absorption,



55-2-118, 254
 136622
 01.5.55

P₂ 28.7 %, K₂D constituted 50 % of the total tannin showed: degree of tannage, P₂ 88.7, K₂D 70.3; water absorption, P₂ 28.7%, K₂D 34.8% (2 hrs.) P₂ 35.7, K₂D 43.3 (24 hrs.). The differences are attributed to the SO₃H groups of K₂D.

SECOND INTERNATIONAL COLLOQUIUM OF LEATHER TECHNOLOGISTS. Miroslav Tomisek (Ministry Consumer Goods Ind., Prague). *Kožarství* 7, 4-6 (1957); *Chem. Abst.* 52, 17770 (1958). The following conclusions were reached: Exchange syntans (I) can be used as part of the total tanning material used for tanning any type of leather. Auxiliary syntans in high amount tend to lower the quality of the leather. Lignosulfonate should not be classed as a tanning material, and production of I from lignosulfonate is not now economically possible. The best raw materials for producing I are monohydroxy phenols, naphthols, and especially pyrocatechol and resorcinol. The tanning properties of I vary inversely with their SO₃H content. This applies also to mixtures of I with vegetable tannins, hence use of sulfited tannins with I is to be restricted. The hide powder method does not give the tannin content of I correctly.

PRODUCTION OF WHITE LEATHER. Vladimír Pekton and Jaroslav Ondráček (Leather and Allied Trades Research Inst., Gottwaldov, Czech). *Kožarství* 7, 287-97, 306-11 (1957); *Chem. Abst.* 52, 17771 (1958). A review. Processes described are: (1) bleached Cr tannage, 1.7% Cr₂O₃, neutralized with Na₂S₂O₃, bleached with H₂C₂O₄ and bleaching syntans; (2) sulfochloride tannage preferably followed by Cr retan; (3) melamine tannage, which may follow a Cr pretannage; (4) Zr tannage. Tannages with Al and HCHO are used only in combination with other tannages. Fat-liquoring with sulfated castor oil and finishing with casein-resin binders and white pigments are recommended.

PROCESSES AFTER TANNING

THE USE OF VEGETABLE OILS IN TANNERIES. Vladimír Pilc (Leather and Allied Trades Research Inst., Gottwaldov, Czech). *Věda a výzkum v průmyslu Kožedělném* 2, 59-68 (1957); *Chem. Abst.* 52, 17771 (1958). A sulfated oil for sole leather made from 70% rapeseed oil (I), 10% sunflower oil (II) and 20% refined fat from glue factories (III) is described. Sulfated I is also suitable for fat-liquoring of suede and calf leathers and for furs, especially rabbit skins. I is sulfated with 25% H₂SO₄ at

20°. Sulfation of a 1:1 mixt. of soya bean (IV) and sperm (V) oils with 25% gives a fat liquor suitable for calf leather. Sulfation of 40% I, 40% II, 20% III after addition of 10% mineral oil gives a good fat liquor for side and pig upper leathers. A mixt. of sulfated and unsulfated II used for greasing pig waterproof leather caused heating and spontaneous combustion. This can be prevented by addition of an antioxidant, e.g. 0.25 to 1.0% of phenyl-2-naphthylamine. The spontaneous combustion occurs only with pig, not with steer-waterproof leather. The reason is not known. Glazed kid fat-liquored with a fat liquor composed of 50% sulfated castor, 25% linseed oil and 25% elain showed no spue after several years.

EXPERIENCE WITH DRYING OF PASTED SIDE UPPER LEATHER. Vladimír Pektor and Lumír Hula (Leather and Allied Trades Research Inst., Gottwaldov, Czech). *Věda a výzkum v průmyslu Kožedělném* 3, 99-118 (1958); *Chem. Abst.* 52, 17771 (1958). Recommended procedures for leather to be pasted include medium liming, short bating, fat liquoring with 15-20% more oil than normal of which at least 20% should be nonsulfated, neutralization to pH 4.5-5.0 in the 'Cut' and retannage with a mixt. of vegetable extract and syntans. The H₂O content before pasting should be 55-60%. Details of drying and reconditioning cycles are given.

TESTING

DETERMINATION OF DEGREE OF SULFITATION OF VEGETABLE TANNING MATERIALS. Antonín Stehlik, Dragošlav Kokes, Miroslava Podolska and Eduard Muck (Leather and Allied Trades Research Inst., Gottwaldov, Czech). *Věda a výzkum v průmyslu kožedělném* 2, 53-8 (1957); *Chem. Abst.* 52, 17769 (1959). Turley's method is too long. A method is proposed for determining free SO₂, SO₂ present as sulfites, and SO₂ bound to tannin (I). Dissolve 50 g. solid sulfited extract in 1000 ml. cold water, add HCl to lower the pH to 2.8-3.1, pipet into a 250 ml. flask, boil under reflux in an atmosphere of N for 1 hr. distil into a 3% soln. of H₂O₂ and titrate the acid formed with 0.1 N NaOH to the bromophenol blue end point (free SO₂). To the residual soln. add 5 ml. concentrated HCl and 10 ml. HCHO (40%) to precipitate the sulfited tannin, boil under reflux 30 min., cool, filter, and wash free from sulfate. Transfer the washed precipitate to a Kjeldahl flask, add 5 ml. fuming HNO₃ and 80 ml. (30%) H₂O₂ and digest to a trans-

parent solution. Det. I by gravimetric determination of SO_4 . A blank determination by using nonsulfited quebracho is recommended. The colloidal ppts. obtained with highly sulfited extracts, can be filtered hot after addition of a 10% solution of AcOK to the suspension. Lignosulfonic acid must be removed before the analysis by precipitation with urea. The I content of sulfited quebracho and spruce extracts are 3.28—3.72 and 2.16–2.58% respectively. The following values of I were obtained for spruce extract sulfited with 1.2 and 1.8% of a 1:1 mixt. of NaHSO_3 , and Na_2SO_3 , and with 2, 3, and 5% Na_2SO_3 : 2.28, 2.96, 2.16, 3.36 and 4.58%.

DETERMINATION OF SYNTANS IN THE PRESENCE OF VEGETABLE TANNINS. *Drohoslav Kokes, Eduard Muck, and Miroslava Podolska (Leather & Allied Trades Research Inst., Gottwaldov, Czech). Věda a výzkum v průmyslu kožedělném 2, 45–51 (1957); Chem. Abst. 52, 17769 (1958).* Method of Bekes is criticized. The consumption of KMnO_4 depends on temp., speed of addition, and concentration and amount of indigo carmine indicator. The titration with use of Pulfrich photometer is described. To 750 ml. H_2O at 23° in a porcelain dish, add 5 ml. of analytical tannin solution and titrate with 0.025 N KMnO_4 (10 ml. a min.) to a distinct lowering of extinction of the soln., with S. 57 filter. A 7.5 ml cuvette is used for determination, and its contents are returned to the dish. Extinction of 0.3 is taken as the end point. The KMnO_4 No. (I) of a tannin equals ml. 0.025 N KMnO_4 for 5 ml. of an analytical tannin soln. Following I have been determined: quebracho 14.04, 13.04; sulfited quebracho 17.09; valonea 16.93; wattle 12.10; chestnut 16.70; spruce 8.41, sulfited spruce 11.00—13.92; Czechoslovak syntans: K_1D 5.47; K_2D 9.45; Kortan Q_1 11.72; BNO 7.58; SN_{25} 7.28. The I cannot therefore be considered as a characteristic value for distinguishing syntans and vegetable tannins. Carry over of dil. lime liquors containing Na_2S to first suspender liquors greatly influences I. By determination of I in 10 lab. suspender liquors with 35% syntans SN_{25} and 65% Quebracho after passage of 5 lots of pelt, a preferential absorption of tannin in suspenders 1–7 and of syntan in suspenders 8–10 has been ascertained. A similar result was obtained with 60% SN_{25} and 40% quebracho. The KMnO_4 method is applicable, if only 2 components are present in tanning liquors, but not with 4 components, as is usual.

A QUANTITATIVE STUDY OF FASTNESS TO WET-RUBBING. C. H. Spiers and J. P. W. Burnett. *J.S.L.T.C.* 43, 38

(1959). The Satra machine has been used to investigate quantitatively the rate of pigment removal from a pigment-finished leather by wet rubbing. It is based upon standardising the moisture content of the felt pads, and changing them every 25 revolutions. The pigment removed per 25 revolutions is found by analysis of the pads.

This technique of following the removal of a constituent of a leather surface coating by estimating the amount transferred to the rubbing pad is obviously capable of extension and can be usefully applied in the study of surface coatings.

In the present work it has been found that

(1) the pigment in a casein-based pigmented finish is removed at a steady rate by wet rubbing, whether the finish is fixed or not, suggesting that pigment removal depends on abrasion and not upon solubilisation of the binder, and that the coating is uniform throughout its thickness.

(2) fixation with formaldehyde/acetic acid, formaldehyde/chrome alum or formaldehyde/chromium acetate considerably improves the fastness the latter being slightly more effective than the others.

(3) ageing beyond two days after fixation has little value in the case of formaldehyde/chromium acetate but ageing 7 days is advantageous with formaldehyde/acetic acid fixation.

(4) plating a leather with casein finish fixed with formaldehyde/acetic acid appears to have damaged the outer layers of the finish and improved the inner layers.

(5) fastness is improved by increasing the proportion of casein binder because more casein binder must be removed to release the same amount of pigment.

(6) application of a clear casein season protects the pigmented film against abrasion.

(7) addition of a resin emulsion to a casein-based pigmented finish apparently produces a layered coating. Addition of a small amount produces an outer abrasive-resistant pigmented resinous layer over the casein-based finish. Addition of large amounts seems to produce an outer pigment-free, resinous layer, beneath which is a resinous pigmented layer which is more resistant to wet rubbing than a casein-based pigmented layer.

OBSERVATIONS OF THE OFFICIAL METHODS FOR THE DETERMINATION OF COLOUR TOLERANCES. H. E. Nurten. *J.S.L.T.C.* 43, 50 (1959). The method is important for works control and development research into the production of dyed and pigment-finished leathers and the manufacture of finishes for leather, provided the volume of work justifies the expense of the colorimeter. Although the Method has been adopted by the Society, it would appear that, in consideration of the following, amendments should be made:

1. To the method of measuring chromaticity and luminance: covering

- (a) the limitations and possible errors;
- (b) a standard procedure for the preparation of samples in the case of suede or printed leathers;
- (c) the use of a secondary standard of greater permanence than magnesium oxide;
- (d) improvement of the accuracy of measurement for samples of low luminance, e.g., by the specification of differential colorimetry.

2. To the method of calculating colour tolerance: covering adjustment of both the chromaticity and luminance data and their interdependence in accordance with acceptable experimental results.

A RAPID METHOD FOR FAT EXTRACTION FROM FATTY TISSUE. V. Piulskaya. *Myasnaya Ind. S.S.S.R.* 29, No. 1, 9-10 (1958). Grind fatty tissue or fatty meat product, weigh a 5-g sample, mix thoroughly with 15 g. anhyd. Na_2SO_4 , transfer the mixture to a 250-300 ml. Erlenmeyer flask, add 25 ml. CHCl_3 , shake the resulting mixture for 1 minute, and separate the liquid phase immediately by filtration. Aliquot 5 ml. of the filtrate for the determination of I no., acid no., or peroxide no.; evaporate another 5-ml sample on a steam bath in a Petri dish and then dry at $100-2^\circ$ to a constant weight in an air oven to determine the amount of fat in the sample. This method of the fat extraction compared favourably with other more tedious methods such as blending in a Waring Blendor, evaporation of CHCl_3 under CO_2 or N stream, etc., without oxidation of the fat during the extraction procedure.

ANALYTICAL CHEMISTRY OF PLASTICS. VIII. Completion and improvement of analysis of polyamides. Elisabeth Schroder (*Inst. Chem. Technol. Plaste, Leipzig, Ger.*). *Plaste u. Kautschuk* 5, 49-51 (1958); *Chem. Abst.* 52, 17781, (1958). Qualitative methods for identification of polyamides, including m.p., mixed m.p., and paper chromatography, were reviewed. "Critical acid concentrations" for HCl and H_2SO_4 were determined for 7 polyamides by adding 0.5-ml. portions of either 35% HCl or 94% H_2SO_4 to a solution (which had been held at 20° for 24 hrs) of 0.5 g. polymer in 10% of the corresponding acid until the solution became clear, then adding measured amounts of H_2O until the solution became cloudy. A method for quantitative analysis of poly hexamethylene adipates (I) was developed by testing a 1:1 salt of $(\text{CH}_2)_6(\text{NH}_2)_2$ and adipic acid on ion-exchange columns. For determination of I, a 1.0000 g. sample was boiled with 2 ml. concentrated HCl 5 hrs. with addition of H_2O to make up 200 ml., and 20 ml. of cooled solution was placed on a column of Wofatit KPS 200 and washed with 300 ml. H_2O . The total acidity was titrated with 0.1 N KOH with phenolphthalein indicator, and the Cl content was determined by the Volhard method. The % I = $(\text{ml. } 0.1 \text{ N KOH} - \text{ml. } 0.1 \text{ N Ag NO}_3) \times 11.3 \times 1000 \text{ mg. dry sample}$. Determinations of the free $(\text{CH}_2)_6(\text{NH}_2)_2$ on columns of Wofatit L150 were not quantitative. *Ibid.* 107-7. The method for I was modified for polycaprolactams (II) by using $\text{NH}_2(\text{CH}_2)_5\text{CO}_2\text{H}$ as a model. The hydrolyzate was placed on a column of Wofatit L150, washed with 10.00 ml. 0.1 N KOH, then 350 ml. H_2O , the eluate neutralized with 0.1 N HCl, CH_2O added, and then titrated with 0.1 N KOH to a phenolphthalein endpoint. The % II = $\text{ml. } 0.1 \text{ N KOH} \times 11.3 \times 1000/\text{mg. dry sample}$. A different modification was made for polymers containing sebacic acid. Elution of the ion-exchange column was with 350 ml. 6:4 MeOH: H_2O saturated with N. In the calcn., the numerical factor was 14.1. Poly (aminoundecanoic acid) (III) was analyzed by hydrolyzing 200 mg. with 2 ml. 35% HCl for 12 hrs. in a special pressure vessel at 130° . The ϵ -amino undecanoic acid obtained was determined by making the solution up to 100 ml., treating 20 ml. of this with 0.1 N KOH to a color change in phenolphthalein, adding 25 ml. formalin, and titrating with 0.05 N KOH. The % III = $\text{ml. } 0.05 \text{ N KOH} \times 9.19 \times 100 \times 5/\text{mg. dry sample}$. For analysis of mixed polymers containing I and II, the method for I was used to give % I, followed by washing 20.00 ml. of the same hydrolysis solution through a Wofatit L 150 column as usual, titrating the eluate with 0.1 N H_2SO_4 to a

phenolphthalein end point, adding 35 ml. neutral formalin, and titrating with 0.1 N KOH. For exact determination, the column was washed again with 10.00 ml. 0.1 N KOH, the washings neutralised, treated with formalin, and titrated. The % II = total ml. 0.1 N KOH or used titrations after formalin additions—ml. 0.1 N H₂SO₄ × 11.3 × 1000/mg. dry sample. For a polymer mixture containing I, II and CO[(CH₂)₂CO₂H]₂, the hydrolyzate was passed through a KPS 200 column, the keto acid was removed by refluxing 3 hrs. with 0.1 N NH₂OH. HCl, and the free HCl was then determined. For determination of end amino groups in polyamides, the 2, 4-dinitrofluorobenzene method of Zahn and Rathgeber was used, but the 2-propynyl alc. solvent was replaced by o-cresol.

THE TITRATION OF ORGANIC BASES IN CHLORO-BENZENE WITH ACETOUS PERCHLORIC ACID. M. Pernarowski and D. W. Blackburn (Purdue Univ. Lafayette, Indiana). *J. Am. Pharm. Assoc.* **47**, 585-9 (1958); *Chem. Abst.* **52**, 16983 (1958). Organic bases can be determined by potentiometric titration in chlorobenzene (I) by using glass and sleeve-type calomel electrodes and a Beckman model GS meter with acetous perchloric acid as the titrant. The optimum concentration of titrant to solution at the end point is approximately 20-100. Bases as weak as caffeine can be determined in I but with a limited degree of accuracy. For organic bases insoluble in I a mixture containing 20% glacial AcOH in I can be used. Neutral red, brilliant green, and methyl red can be used as indicators, but bromophenol blue is the most satisfactory.

TECHNICAL IMPROVEMENT OF TWO-DIMENSIONAL PAPER CHROMATOGRAPHY. U. Vetsch and H. Luthi (Versuchsanst. Ostbau, Wädenswil, Switz). *Mitt. Gebiete Lebensm. u. Hyg.* **49**, 29-35 (1958); *Chem. Abst.* **52**, 16971 (1958). The test substances are spotted on the paper on 3 or more points in 7-cm. distance on a line. After development of the chromatogram the paper is cut in strips parallel to the line of development, 1 strip is used to make the chromatogram visible, and the corresponding spots on the other strips, after being fastened to another sheet of paper, are developed with a 2nd solvent.

A SEMI-AUTOMATIC MULTI-PIPETTING DEVICE FOR PAPER CHROMATOGRAPHY. J. W. Parteons. *Journ. Chromat.* **2**, 58 (1959). A semi-automatic multi-pipetting device is described

which has proved useful in paper chromatography, particularly where it has been necessary to apply large volumes of a solution or a large number of different solutions to one or more chromatographic papers for one or two dimensional development. Any length of paper and any width up to 60 cm can be accommodated; the distance between starting points can be varied at will within wide limits; pipettes can be removed individually at any time for cleaning or refilling and the papers can be changed without disturbing the rest of the apparatus; provision is made for hot or cold air drying of the solutions applied to the paper.

A GENERAL METHOD FOR DETECTION AND RECORDING OF COMPONENT BANDS IN CHROMATOGRAPHY WITH LIQUID ELEMENTS. James C. Sternberg and Lewis M. Carson. *Jour. Chromat.* **2**, 53 (1959). A method for the detection and recording of component bands has been described. It was found to be sufficiently general to insure its applicability to a wide variety of solvent-solute systems, and since it senses the column effluent it is independent of the type of chromatographic column used. It appears to be readily adaptable to the quantitative estimation of non-volatile solute bands for which it is based on essentially a colligative property, and it appears applicable, with specific calibration, to volatile solute bands as well.

THEORIES

SOLUTION PROPERTIES AND CONFIGURATIONS OF A POLYAMPHOLYTIC POLYPEPTIDE: COPOLY-L-LYSINE-L-GLUTAMIC ACID. P. Doty K. Imahori, and E. Klemperer (Harvard Univ.) *Proc. Natl. Acad. Sci. U.S.* **44**, 424-31 (1958); *Chem. Abst.* **52**, 17349 (1958). Equimolar copolymers of L-lysine and L-glutamic acid (mol. wts. about 40,000) exhibit the α -helical configuration to the extent of 15 % in neutral 0.15 M NaCl. The amount increases to 50 % at pH 4 and vanishes at pH 12 or upon removal of the salt. This behaviour is explained by the observation that under comparable conditions the poly-L-lysine helix is weaker than the poly-L-glutamic helix. Two apparent metastable states are observed in copolymers not exposed to alkaline pH, one of which is in the β -configuration. Urea addition causes denaturation similar to proteins. In chloroethanol, the configuration is completely helical. Addition of trifluoroacetic acid converts the polypeptide to the randomly coiled form. This solvent system permits determination of helix content by infrared spectra.

STUDY OF N-TERMINAL GROUPS OF ZEIN OF DAGES-TAN VARIETY OF CORN. N. N. Mal'teva and M. S. Reznichenko (*Inst. Soviet Trade, Leningrad*). *Biokhimiya* **23**, 372-6 (1958); *Chem. Abst.* **52**, 17350 (1958). N-terminal groups were determined by preparation of the dinitrophenyl derivs. of the amino acids, followed by chromatographic analysis. The N-terminal groups consisted of threonine, and aspartic and glutamic acids.

BIOGENESIS OF CONDENSED TANNINS FROM LEUCO-ANTHOCYANINS. David G. Roux (*Leather Ind. Research Inst., Grahamstown, S. Africa*). *Nature* **181**, 1454-6 (1958); *Chem. Abst.* **52**, 17422 (1958). Examination of the sapwood of *Schinopsis quebracho-colorado* by paper chromatography revealed the presence of a monomeric leucofisetinidin in high concentration. From 200 g. of the cross-section of a sapling of this tree containing only a trace of black heartwood, 3.4 g. of leucofisetinidin could be isolated, forming about 10 % of the total phenolic extractives present in the sapling. The chromatograms show that the monomeric leucofisetinidin gradually disappears, from the outer sapwood where it is present in highest concentration, towards the central pith. Apart from the isolation and presence of leucofisetinidin, this work provides presumptive evidence of its transformation in the sapwood, to the various polymeric forms present in the heartwood. Evidence of apparent secondary condensation of polymers of intermediate dimensions to those more highly condensed forms, which constitute a high percentage of quebracho extract is also provided. The leucofisetinidin, 7, 3', 4'-trihydroxyflavan-3, 4-diol, can therefore be regarded as the possible precursor of quebracho extract. The apparent transformation of monomeric (non-tannin) leucoanthocyanins into polymeric leuco-anthocyanins (or tannins) in the case of *S. quebracho-colorado* and *Acacia melanoxylon*, and also the co-existence of leucoanthocyanins of varying complexity but of similar basic structural pattern in the above and other species examined is highly significant.

BYE-PRODUCTS

BONE SOAKING BEFORE EXTRACTION OF GLUE. Z. V. Khokhlova and P. D. Mernenko (*All-Union Sci. Research Inst. Meat Ind., Moscow*). *Myasnaya Ind. S.S.S.R.* **29**, No. 1, 52-3 (1958); *Chem. Abst.* **52**, 17772 (1958). Soaking of bones in 1 % solns. of $ZnSO_4$ or MgO for 24 hrs. under atmosphere pressure, or for 4 hrs. under hydraulic pressure of 10-12 kg./sq. cm., was

found to be beneficial for the subsequent water-steam extraction of the glue from the bones and the glue quality (relative viscosity and gluing power). $ZnSO_4$ solvent, owing to the presence of free SO_4 and its low pH (5.5-6.5), greatly depressed the growth of micro-organisms during the soaking process.

PREPARATION OF GLUTAMIC ACID FROM GLUE. Raul Cornejo Aleman. *Anales fac. farm. Y bioquim., (Univ. nacl. mayor San Marcos, Lima)* **6**, 581-7 (1955); *Chem. Abst.* **52**, 17772 (1958). Glutamic acid (I) was obtained as its hydrochloride from domestic and imported carpenter glues by hydrolysis with HCl soln., and isolation through saturation of the hydrolysis soln. with HCl. A modification was introduced by treating the solution in which I has crystallized, with an equal vol. of acetone to make separation of the crystals easier.

PATENTS

SILICA TANNING. Paul E. Chambard and Institut de recherches pour les industries du cuir. *Fr.* **1,034,227**, July 21, 1953; *Chem. Abst.* **52**, 17772 (1958). Hides are tanned by treatment with a bath containing an alkali metal metasilicate and a mixt. of a strong and a weak acid at pH 3.2. The stable SiO_2 sol having a well defined minimum particle size, plus addition of a surface-active agent, assure good penetration to give thoroughly tanned, fine, supple, soft leather. For example calf skins were pickled for 24 hrs. in a bath containing com. NaCl 6, 100 % AcOH 7.5, commercial HCl 1 and water 100 kg. per 100 kg. of hides. The bath had a pH of 3.2-3.3. After 8-10 hrs. standing, tanning was begun in a bath containing $Na_2SiO_3 \cdot 5H_2O$ 14.7, 100 % AcOH 7.5, commercial HCl 16, and water 200 kg. (pH 3.2). After 1 hr. 1 kg. of a fatty emulsion was added. Tanning was continued for 36 hrs. and repeated in an identical bath. The skins were then neutralized to pH 5, rinsed, treated with a fatty emulsion, dried, and finished.

TREATMENT OF CHROME-TANNED LEATHER WITH DEACIDIZING AGENTS. Bohme Fettchemie G. m.b.H (*Friedrich Schmitt and Adrianus Schenkels, inventors*). *Ger.* **937,371**; *Chem. Abst.* **52**, 17772 (1958). Mixts. of the alkali metal, alk. earth, or NH_4 salts of low molecule organic acids (I) obtained by the neutralization of paraffin oxidation products are suitable for deacidification of chrome-tanned leather. I consist largely of a mixt. of HCO_2H , AcOH, and propionic acid with aldehydes and alcohols.

Usual deacidizing agents, e.g., water-soluble sulfites, bisulfites, or thiosulfates, can also be used. The leather treated by this process has a high Cr_2O_3 content in each layer. For example, leather was treated with 250 % water and 2 % I for 45 min. at 30°.

BLEACHING AND CLEANING OF LEATHER. *Zellstoff-Fabrik Wald-hof* (August W. Sohn and Ernst Roser, inventors). **Ger. 935,991**, Dec. 1 1955; *Chem. Abst.* 52, 17772 (1958). Leather is cleaned, bleached, and detanned with aq. solutions of chlorites (preferably the Na or Ca salt) or with ClO_2 solutions. Complete detanning is achieved if the leather is treated with slightly acid 30-50 % NaClO_2 solutions, and peltlike leather is obtained. Leather tanned with HCHO cannot be detanned but can be bleached to give white leather of improved toughness and dyability. Leather tanned with synthetic sulfonated tanning agents must be bleached with less concentrated solutions. If a vegetable-tanned leather is bleached with an alkaline solution of a chlorite, the leather is treated subsequently with an acid, e.g., HCOOH , in order to prevent the detanning of the inner parts of the leather. Thus, leather tanned with a combination of vegetable and lignin tanning agents was treated with a solution of 10 Kg. NaClO_2 in 200 l. water at 30°. After wetting, dil. HCOOH (1% by vol.) was added. The leather was bleached for 10-15 min. and then put in a bath which was slightly acidified with HCOOH , sammed, treated with bleaching oil, and dried slowly to give uniformly bleached leather. In a series of tests, the bleaching action of NaClO_2 was compared with that of oxalic acid and of HCl .

DRESSING OF LEATHER. *Farbenfabriken Bayer A-G* (Wilhelm Graulich, Gustav Mauthe, and Kurt Eitel, inventors). **Ger. 929,209**, Aug. 1, 1955. Emulsions of buta-diene-acrylonitrile copolymers are produced with so much modification that they do not form stable films with any structural viscosity. The viscous oils may be used as a bottom coating from strongly fluffed, highly adsorbing leather without producing a coarser grain. On compounding with emulsions of polymers of greater strength, coatings without tackiness are obtained. The copolymer should contain 50% acrylonitrile. A 3rd component, such as styrene, an acrylate, or 1, 1-dichloroethene, can be copolymerized with the other components. Thus, 4 parts of the Na salt of a long-chain paraffin sulfonate, 0.05 part $\text{K}_2\text{S}_2\text{O}_8$ were dissolved in 180 parts water. To this solution, 60 parts butadiene and 40 parts acrylonitrile containing 6 parts diisopropylxanthogen disulfide were emulsified in an autoclave without additional gas pressure. Then, 0.25 part triethano-

lamine was added; the mixture was polymerized at 40° until 80% conversion was achieved. If the polymerization stopped before 80% conversion it was started again by addition of $\text{K}_2\text{S}_2\text{O}_8$. The 30% emulsion obtained was stabilized by addition of dispersion of 1, 5-di tert-butyl p-cresol (1% by wt. of polymer). Similarly butadiene 50, acrylonitrile 40, styrene 10, and dodecyl mercaptan 5 parts were copolymerized to 100% conversion and stabilized. A fluffed, semichrome leather was treated with a mixture of 100 parts of a comp. casein-base pigment finish dye paste, 100 parts of the above butadiene-acrylonitrile co-polymer, 4 parts concentrated aniline dye, and 300 parts water to give a level surface without cracks. Subsequently, a plush coating could be applied.

TREATMENT OF WASTE CHROME LEATHER. *Elektrochemische Fabrik Kempen-Rhein, Dr. Brandenburg & Weyland, G.m.b.H.* (Fritz Hess and Wilhelm Lenz, inventors). **Ger. 937,545**, Jan. 12, 1956; *Chem. Abst.* 52, 17774 (1958)—Before cooking to obtain glue, the wastes are dechromed with the aid of a mineral acid. The product is then washed with a solution of a Mg. salt and boiled with a solution, which is weakly basic because of its content of MgO . The concentration of acid and salts is 3%.

WAXES FOR TREATMENT OF LEATHER AND FLOORS. *H. Schneider & Co.* (Hans Schneider, inventor). **Swiss 323,323**, Sept. 14, 1957 (cl.). 37g. *Chem. Abst.* 52, 17762 (1958)—Polysiloxanes in the form of silicone oils (I) are added to wax components (II) to enhance their water resistance and improve luster. Examples of II for leather and floor coverings, respectively are: Wax, 22, 22; terpene oil 64, 38; dye 1, 0; water 10, 38; I 2, 1; preservative, 0.2, 0.2; and perfume, 0.8, 0.8%.

STUDY OF N-TERMINAL GROUPS OF ZEIN OF DAGES-TAN VARIETY OF CORN. N. N. Mal'teva and M. S. Reznichenko (*Inst. Soviet Trade, Leningrad*). *Biokhimiya* **23**, 372-6 (1958); *Chem. Abst.* **52**, 17350 (1958). N-terminal groups were determined by preparation of the dinitrophenyl derivs. of the amino acids, followed by chromatographic analysis. The N-terminal groups consisted of threonine, and aspartic and glutamic acids.

BIOGENESIS OF CONDENSED TANNINS FROM LEUCO-ANTHOCYANINS. David G. Roux (*Leather Ind. Research Inst., Grahamstown, S. Africa*). *Nature* **181**, 1454-6 (1958); *Chem. Abst.* **52**, 17422 (1958). Examination of the sapwood of *Schinopsis quebracho-colorado* by paper chromatography revealed the presence of a monomeric leucofisetinidin in high concentration. From 200 g. of the cross-section of a sapling of this tree containing only a trace of black heartwood, 3.4 g. of leucofisetinidin could be isolated, forming about 10 % of the total phenolic extractives present in the sapling. The chromatograms show that the monomeric leucofisetinidin gradually disappears, from the outer sapwood where it is present in highest concentration, towards the central pith. Apart from the isolation and presence of leucofisetinidin, this work provides presumptive evidence of its transformation in the sapwood, to the various polymeric forms present in the heartwood. Evidence of apparent secondary condensation of polymers of intermediate dimensions to those more highly condensed forms, which constitute a high percentage of quebracho extract is also provided. The leucofisetinidin, 7, 3', 4'-trihydroxyflavan-3, 4-diol, can therefore be regarded as the possible precursor of quebracho extract. The apparent transformation of monomeric (non-tannin) leucoanthocyanins into polymeric leuco-anthocyanins (or tannins) in the case of *S. quebracho-colorado* and *Acacia melanoxylon*, and also the co-existence of leucoanthocyanins of varying complexity but of similar basic structural pattern in the above and other species examined is highly significant.

BYE-PRODUCTS

BONE SOAKING BEFORE EXTRACTION OF GLUE. Z. V. Khokhlova and P. D. Mernenko (*All-Union Sci. Research Inst. Meat Ind., Moscow*). *Myasnaya Ind. S.S.S.R.* **29**, No. 1, 52-3 (1958); *Chem. Abst.* **52**, 17772 (1958). Soaking of bones in 1 % solns. of $ZnSO_4$ or MgO for 24 hrs. under atmosphere pressure, or for 4 hrs. under hydraulic pressure of 10-12 kg./sq. cm., was

found to be beneficial for the subsequent water-steam extraction of the glue from the bones and the glue quality (relative viscosity and gluing power). $ZnSO_4$ solvent, owing to the presence of free SO_4 and its low pH (5.5-6.5), greatly depressed the growth of micro-organisms during the soaking process.

PREPARATION OF GLUTAMIC ACID FROM GLUE. Raul Cornejo Aleman. *Anales fac. farm. Y bioquim., (Univ. nacl. mayor San Marcos, Lima)* **6**, 581-7 (1955); *Chem. Abst.* **52**, 17772 (1958). Glutamic acid (I) was obtained as its hydrochloride from domestic and imported carpenter glues by hydrolysis with HCl soln., and isolation through saturation of the hydrolysis soln. with HCl. A modification was introduced by treating the solution in which I has crystallized, with an equal vol. of acetone to make separation of the crystals easier.

PATENTS

SILICA TANNING. Paul E. Chambard and Institut de recherches pour les industries du cuir. *Fr.* **1,034,227**, July 21, 1953; *Chem. Abst.* **52**, 17772 (1958). Hides are tanned by treatment with a bath containing an alkali metal metasilicate and a mixt. of a strong and a weak acid at pH 3.2. The stable SiO_2 sol having a well defined minimum particle size, plus addition of a surface-active agent, assure good penetration to give thoroughly tanned, fine, supple, soft leather. For example calf skins were pickled for 24 hrs. in a bath containing com. NaCl 6, 100 % AcOH 7.5, commercial HCl 1 and water 100 kg. per 100 kg. of hides. The bath had a pH of 3.2-3.3. After 8-10 hrs. standing, tanning was begun in a bath containing $Na_2SiO_3 \cdot 5H_2O$ 14.7, 100 % AcOH 7.5, commercial HCl 16, and water 200 kg. (pH 3.2). After 1 hr. 1 kg. of a fatty emulsion was added. Tanning was continued for 36 hrs. and repeated in an identical bath. The skins were then neutralized to pH 5, rinsed, treated with a fatty emulsion, dried, and finished.

TREATMENT OF CHROME-TANNED LEATHER WITH DEACIDIZING AGENTS. Bohme Fettchemie G. m.b.H (Friedrich Schmitt and Adrianus Schenkels, inventors). *Ger.* **937,371**; *Chem. Abst.* **52**, 17772 (1958). Mixts. of the alkali metal, alk. earth, or NH_4 salts of low molecule organic acids (I) obtained by the neutralization of paraffin oxidation products are suitable for deacidification of chrome-tanned leather. I consist largely of a mixt. of HCO_2H , AcOH, and propionic acid with aldehydes and alcohols.

NOTES & NEWS

POLYMERS MADE EASILY

A longstanding need for a simple process to prepare condensation polymers may be satisfied by interfacial condensation. Compared to melt polycondensation, the process uses simpler open equipment and requires conditions more nearly atmospheric. Interfacial polycondensation is a versatile laboratory method to make many new condensation polymers in a minimum of time. Even stirring is not necessary, although helpful. It is an irreversible polymerisation using monomers such diamines or diacid chlorides in a two phase system. Variables of interfacial polycondensation affect the final polymer as well as the reaction. The interface provides, through solubility differences, controlled introduction of the aqueous phase reactant into an excess of the diacid halide in the organic phase next to the interface. Upon phase contact both reactants and solvents tend to become partitioned with the opposing phase. A film of high polymer forms at the interface. Strongly coherent when pulled out of the interface, the film forms continuously as an endless cord.

Chem. & Eng. News 36, No. 37, 52 (1959)

SALTS DETERMINED IN NONAQUEOUS SOLUTION

Tetrabutylammonium hydroxide, a recently developed reagent may open the door to a more versatile, convenient method for determining salts in nonaqueous solutions. Quaternary ammonium hydroxides have proved useful for titrating acids in nonaqueous solutions. Among their advantages are: Acidic groups on the same molecule or in acid mixtures can be differentiated, precipitates are seldom formed in titration; glass-calomel electrodes can be used for potentiometric work.

The procedure to determine salts is as follows: The salt is acidified with an excess of sulphuric acid. The liberated acid and unreacted sulphuric acid are titrated differentially. The cation precipitates as a sulphate when a non-aqueous solvent is added. The rest of the analysis is merely a differentiating potentiometric

titration of the liberated and added acids. Pyridine is preferred but acetone also is useful.

This procedure can be used to analyse a broad range of salts including nitrite nitrate mixtures. The chief restriction is that the salt undergoes an anion exchange reaction with the acidifying reagent. Only a simple filtration is needed to remove the precipitate. Solubility difficulties are minimised. Carbonate and hydroxide impurities do not interfere with the determination as they may with some other procedures. Salts of strong acids and weak organic bases can be titrated directly with the reagent without first being acidified.

Chem. & Eng. News 36, No. 37, 55 (1959)

DECEMBER EXPORT OF GOATSKIN FROM CALCUTTA

During December, 1958, approximately 1,200,000 pieces Indian goatskins were exported from Calcutta (compared with about 426,000 for the same period last year, comprising:

Dry-salted: About 1,042,000 of which about 917,000 were for the U.K. and continent and about 125,000 for the U.S.A.

Wet-salted: About 175,000 of which about 33,000 were for the U.K. and continent and about 142,000 for the U.S.A.

Kidskins: About 3,000 to the continent. Of the overall total about 797,000 pieces were for "Iron Curtain" countries.

Leather Trades' Rev., 4-2-1959.

L.T.R. & E.I. TANNED SKINS

In the last week's L.T.R. was a full report of the list of items of leather and tanning materials which, under the new French measure of trade liberalisation, can now be imported into France without quantitative restriction. This was the official list as it was at the time of going to Press and it did not include E.I. tanned goat- and sheepskins. These are now included and the French raw hide import business has returned to trading conditions ruling before World War II. The Government's Hides and Skin Import Agency—Groupement d'Importation des Cuirs et Peaux Brutes—has stopped all operations which it handled since its formation during the war.

The new facility for hide importers will give French tanners a number of advantages. They will be able to buy where and when they want instead of awaiting the release of credits. Business will be facilitated. The granting of import licences through the import agency required about three weeks at least. Now importers can obtain their licences in less than a week.

The disappearance of the agency will cancel the special duty levied on hide and skin imports to finance the agency's operations and the French hide and skin market will now be more closely linked to international price levels.

However, although the import agency has stopped operations, official notification of its suspension has not yet been released. The relevant Government service has not yet issued any decision on this subject and the agency's board did not meet to decide on its liquidation.

Leather Trades' Rev. 21-1-1959

NEW AND SIMPLE-TO-OPERATE SALT CLEANING PROCESS DISCOVERED

In South Africa, the price of industrial salt is very much higher than in England. This high price coupled with recurrent salt shortages, have in the past led to the re-use of salt in curing hides. Dr. A. J. Rossi of South Africa, has developed a salt cleaning process, making the re-use of salt a more satisfactory proposition.

The principle of the process is to wash used salt in a saturated solution of brine. The brine removes blood, soluble proteins, hair, etc., from the dirty salt without reducing the crystal size. The patented floating cone-separator which removes the washed salt from the dirty brine, ensures the smooth working of the process. On leaving the separator the brine is pumped to a storage tank and treated with an alkaline flocculating agent which precipitates the dissolved proteins and blood removed from the salt. At the same time the flocculent accelerates the settling out of suspended dirt and other insoluble matter. After standing overnight, the clear brine is ready for use again and is run off leaving a sludge which is quite dense and easily removed from the bottom of the settling tank.

A great advantage claimed for the process is that antiseptics can be added to the brine as it is put back into circu-

lation and that if the correct type is used sufficient adheres to the washed salt to render it sterile. As the brine is made alkaline, to aid flocculation, it is obviously essential to avoid antiseptics such as zinc chloride. Quaternary ammonium compounds which are antiseptic wetting agents are suggested as the most suitable ones.

—*Leather Trades' Rev.*: 25-2-1959

NEW MEASURE FOR STANDARDISING LEATHER BREAK WITH THE AID OF A GRAINOMETER

Break is the stream of wrinkles formed when the vamp of the shoe is flexed during walking. Since the frequency of the wrinkles relates to durability, appearance and over all grade of leather used in footwear, it is essential that this quality be carefully evaluated. The need is especially emphasised by the failure of traditional, visual, subjective inspection procedures, to define or curb the use of coarse leathers in footwear.

The work of M. Benley of the U.S. Navy Clothing & Textile office in Brooklyn has resulted in the development of an instrument called a leather grainometer which helps to define break objectively.

Leather Trades' Review: 25-2-1959.

WASH'N WEAR LEATHER

"Scotchgard" is a fluoro-chemical developed by Minnesota Mining & Manufacturing Co., for the impregnation of various materials including leather. It enables the leather to resist soil, water and oil without altering it in any way. Wet shoes containing Scotchgard impregnated leather dry, soft and mellow instead of becoming stiff and brittle. The new treated leather makes a wash'n wear leather shoe a reality.

—*Leather & Shoes*: Feb. 14, 1959.

TRADE PROSPECTS WITH FOREIGN COUNTRIES

Leather and Leather Manufactures

Total imports of leather and manufactures from all source into the United Kingdom were at a lower level during January—November, 1958 amounting to £18,003,239 compared to

£21,314,691 during January-November, 1957. This also affected imports from India which amounted only to £8,765,874 (48.7% of the total) during January—November, 1958 compared to £12,296,908 in 1957.

Raw Wool: Prospects Better—Imports of raw wool into the Kingdom from India were at a much reduced level amounting to of the total) during January—November, 1958, compared to £4,238,201 in January—November, 1957.

Indian raw wool prices remained firm throughout the year 1958 due to a large purchase of 27,000 bales by Russia during the year. The prospects for 1959 of export of Indian wool to the United Kingdom are fairly bright provided prices remained competitive in the United Kingdom market. The carpet trade in the United Kingdom is doing exceptionally well and Indian wool is particularly suitable for its requirements. Our Inspection Scheme seems to have worked very satisfactorily and there has been gradual improvement in the quality exported. There is, however, a wide-spread feeling here that if the Government of India established a testing house on the lines of the one at Karachi, the improvement in quality would be even greater with consequent benefit to our export trade.

Suedes of sheep and goat skins in demand

Sweden is one of the largest importers of processed semi and full chrome tanned suedes for the manufacture of garments and raw-skins for tanning indigenously.

Whilst the quality of Indian goat skins has always been well recognised, the Swedes have so far carried the impression that India could not produce tanned suedes of the required quality. Thanks to the light leathers and chrome tanners of Madras, this impression appears to have been removed to a great extent. In the matter of colours, some shortcomings are still stated to be prevalent. Recently Shri Srinivasan of Madras visited Sweden and received a visual knowledge of the processing of suede leathers at the various Swedish tanneries. It is hoped that the shortcomings will now be removed, and India could export this commodity in larger quantities in 1959. Unfortunately, production capacity of good India tanneries is rather low and should be raised without losing time. In the latter part of 1958 it is learnt that imports consisted mostly of the finished suedes as against salted and raw products. The total import of hides and skins

in Sweden in 1957 was of the tune of 83.9 million crowns and in 1958 70.3 million crowns. India, it will be seen, has improved its exports from 1.1 million crowns to 1.2 million crowns during ten months under review. From the statistics given it will be noticed that whilst the increase in value was only of 100,000 the percentage of India's share in the total imports of Sweden is somewhat higher in view of sizeable decline in Swedish imports in 1957. The business done in this commodity is on letter of credit basis.

Sp. Suppl. Jour. Ind. Trade: April 1959.

STUDENTS' ASSOCIATION OF THE INSTITUTE OF LEATHER TECHNOLOGY, MADRAS

The annual day of the students association of the Institute of Leather Technology, Madras was celebrated on 18-4-1959. Mr. J. G. Abraham, B.E., M.I.E. (Ind.) Am. Soc. C.E., Director of Technical Education presided on the occasion.

The student-President, Sri A. K. Natarajan, welcomed the gathering.

Sri B. Venkata Reddi, Principal of the Institute presented the annual report for the year 1958-59. In the course of the report the Principal pointed out that eminent men in the leather field, in the various states hailed from this Institute, including the present Head of the Central Leather Research Institute.

The messages received on the occasion were next read out and prizes were distributed to the various prize-winners.

Haji Maulvi M. Nazir Hussain, member of the Indian Trade Delegation to the Federal Republic of Germany next spoke stressing the need for establishing a National Laboratory in South India to promote leather finishing industry; establishment of such a laboratory alone could improve the quality of finishes.

In his presidential address Mr. Abraham assured that in spite of several handicaps experienced at present by the Institute, all facilities would be provided for improvement at the Institute.

COLONEL AMIR CHAND PRIZES FOR MEDICAL RESEARCH

Lieut.-Colonel Amir Chand, lately Principal of Lady Hardinge Medical College, New Delhi, has donated Rs. 50,000 to the

Indian Council of Medical Research for creating a prize Fund. From the interest earned by this sum, prizes are to be awarded for the best published research work in medical sciences. The Governing Body of the Council has constituted a Trust, called the "Colonel Amir Chand Trust" for the administration and management of the Fund.

The prizes are awarded annually on an All-India basis for the best published research work in any subject in the field of medical sciences including clinical research. The term, "Clinical Research" covers research into the mechanism and causation of disease and its prevention and cure, and includes work on patients in hospitals, field studies in epidemiology and social medicine and observations in general practice.

It has been decided to award *four prizes* in 1959, each of Rs. 300, to graduates of not more than ten years standing for the best research papers in medical sciences published by them during 1958. These prizes will be known as "Shakuntala Amir Chand Prizes".

Both medical and non-medical graduates are eligible for the prizes.

Selections for the award of the prizes will be made by a Selection Board.

In the case of joint authorship of a publication, the prize shall be divided between the authors in such proportion as the Selection Board may decide. The role of the person who applies for the prize should be clearly indicated so as to make it easy to determine whether the major part of the work has been done by that person.

The award of the prizes will be announced at the annual meetings of the Council's Scientific Advisory Board and Advisory Committees in November/December, 1959.

Candidates for award of prizes are required to submit 10 reprints of their papers published during 1958. These should be sent to the *Director, Indian Council of Medical Research, P.O. Box No. 494, New Delhi*, so as to reach him *not later than 1st August, 1959*. The papers should be accompanied by a short biographical sketch and two copies of passport-size photographs of the candidate/candidates concerned.

RESEARCH BEARS FRUIT

Practical Demonstration No. 6

3rd Series: Manufacture of Chrome/vegetable tanned
'Crushed Kid'

Period: 15th June, '59 to 6th July, '59.

Place: Central Leather Research Institute, Madras-20.

Crushed kid leather is manufactured from goat skins by chrome vegetable tanning and is used for making high-class foot-wear, handbags, fancy articles, etc. This type of leather is manufactured as an allied line of the glazed kid industry. The demand for leather foot-wear and fashion wear in India is ever increasing and so there is scope for this type of leather. This field could be profitably exploited by the Indian tanners, especially, by the Glazed kid and Upper leather manufacturers and hence a process for this has been worked out at this Institute.

It is proposed to demonstrate this process from 15th June, to 6th July, 1959. If you are interested in this line of work, you are requested to send representative/s to get trained in manufacturing this type of leather. You may kindly intimate the name/s, of the representative/s likely to attend this demonstration.

BYE-PRODUCTS UTILISATION

The subject for the next symposium to be held at the Central Leather Research Institute, in February 1960, will be "Utilisation of BYE-PRODUCTS".

52
M97, Jan 21/59, 154
259.5-10

C. L. R. I. NEWS

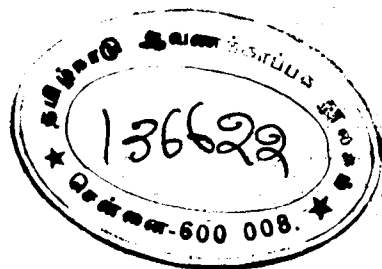
Dr. Y. Nayudamma, Director, Central Leather Research Institute, Madras has been nominated as Principal member of the Advisory Committee for Leather and Tanning Industry of the U.P. including the U.P. Government Pilot Project for Footwear at Agra.

Dr. S. K. Barat, Assistant Director, Central Leather Research Institute, Madras, has been nominated as Alternate member of the Advisory Committee for Leather and Tanning Industry of the U.P. including the U.P. Government Pilot Project for Footwear at Agra.

Dr. Y. Nayudamma, Director, Central Leather Research Institute, Madras has been elected as a member of the Technological Research Sub-Committee of the Indian Central Arecanut Committee for 1959-60.

Dr. Y. Nayudamma, Director, made a study tour of Kawnpur region between 21st and 26th April, 1959.

His Excellency Mr. Trampeyzski, Polish Minister of Foreign Trade, accompanied by a party of 5 visited the Institute on 29th April, 1959.



"Registered No. M. 7050."

CLRI Publications

PROCESS BULLETIN No. 1: Adapted processes for the manufacture of box and willow sides—Re. 1/- per copy. Postage extra.

PROCESS BULLETIN No. 2: Processes for the manufacture of Glazed kid. Re. 1/- per copy. Postage extra.

PROCESS BULLETIN No. 3: Manufacture of Roller skins. Re. 1/- per copy. Postage extra.

PROCESS BULLETIN No. 4: Fish Oil of the West Coast for the Indian Leather Industry. Re. 1/- per copy. Postage extra.

PROCESS BULLETIN No. 5: Rapid Tannage of Sole Leather. Re. 1/- per copy. Postage extra.

SYMPOSIUM ON EAST INDIA TANNING INDUSTRY AND TANNING AGENTS (1955): Proceedings, discussions and papers presented. Re. 1/- per copy. Postage extra.

SYMPOSIUM ON "RAW HIDES & SKINS"—Curing and Preservation (1957): Proceedings and Papers. Re. 1/- per copy. Postage extra.

SYMPOSIUM ON "LEATHER AUXILIARIES" (1958): Proceedings and Papers. Re. 1/- per copy. Postage extra.

SYMPOSIUM ON "TANNING AS A SMALL SCALE & COTTAGE SCALE INDUSTRY" (1959): Proceedings and Papers. Re. 1/- per copy. Postage extra.

For further information please contact

Director

CENTRAL LEATHER RESEARCH INSTITUTE

Adyar, Madras-20, India
