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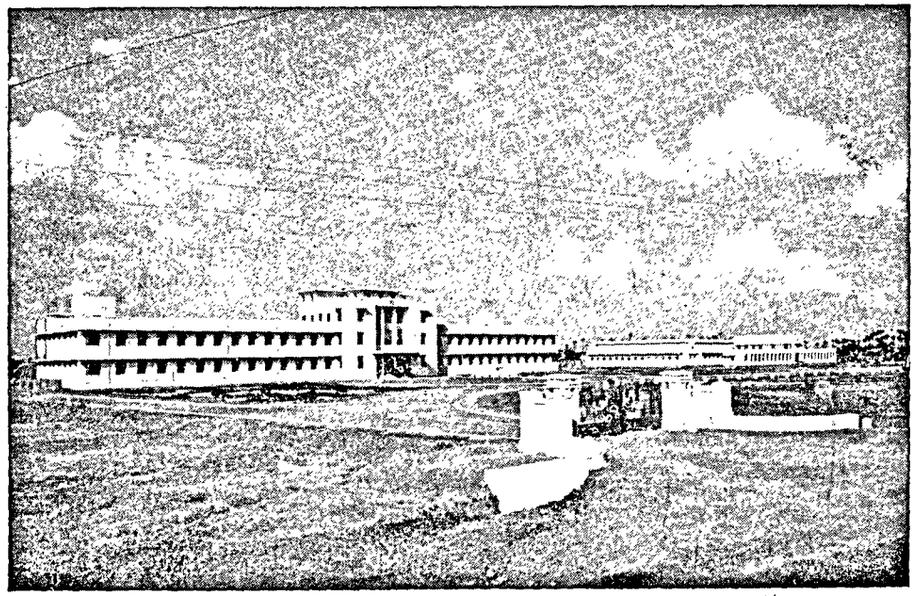

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STANDARDISATION AND EVALUATION OF DIFFERENT METHODS FOR THE QUANTITATIVE ESTIMATION OF PROTEASE ACTIVITY

S. M. BOSE AND W. MADHAVA KRISHNA

SUMMARY

1. With a view to standardise a routine method for the quantitative estimation of proteolytic activity of different types of proteases viz., trypsin, pepsin, papain and "Calotropain", a number of methods, viz. (a) alkali-titration, (b) formol-titration, (c) Lohlein-Volhard, (d) Gross-Fuld, (e) Gelatin-viscosity, (f) Non-protein nitrogen and (g) colorimetric method with phenol reagent were adopted with suitable modifications.

2. Two colorimetric methods making use of (i) α-nitroso-β-naphthol and (ii) Millon's reagent were developed for the quantitative estimation of the proteolytic activity of the enzymes.

3. The merits and demerits of each of the methods tried were discussed. The colorimetric method with the phenol reagent was found to be the most suitable for routine quantitative estimation of proteolytic activity.

Proteolytic enzymes find wide application in various industries and in pharmaceutical preparations. They are widely used as enzyme bates for modern leather manufacture. It is also known that proteolytic enzymes are used for unhairing skins and hides for the manufacture of leather. A routine method for the quantitative estimation of proteolytic activity is essential for any study related to the application of proteases in leather or any other industry. Although different methods are available for the determination of protease activity, these methods have their own limitations and hence cannot be applicable universally. Some of these methods are also known to have certain drawbacks with regard to accurate quantitative estimation. It was, therefore, thought desirable to modify and standardise the available methods and to study their suitability for routine quantitative estimation of proteolytic activity of different types of the protease.

EXPERIMENTAL

The activity of different types of the proteolytic enzyme, viz. trypsin (E. Merck), pepsin (E. Merck), papain (E. Merck) and a protease which was isolated[1] from the latex of madar plants

(*Calotropis gigantea*) by acetone precipitation method and which was named as "Calotropain" was determined by different methods. The enzyme solution was always prepared fresh by dissolving a known weight of the enzyme in measured volume of glass-distilled water and its action was tested against suitable substrate. The pH of the substrate solution was adjusted by addition of dilute acid or alkali as per the optimum pH for the reaction of the enzyme under test. For trypsin, the pH used was 8.0-8.5, for pepsin 2.0-2.5, for papain 5.0-6.0 and for calotropain 4.0-4.5. The following methods were tried with suitable modifications in order to standardise them for routine quantitative determination of protease action. Each estimation was repeated after different periods of hydrolysis of the protein by adopting the same procedure in order to see whether these methods would record the small difference in the degree of proteolysis.

I. ALKALI TITRATION METHOD.

This method was first devised by Willstatter and Waldschmidt — Leitz [2] and later modified by Macrae. [3] According to the method which was further modified and followed by us, 12 c.c. of 10 per cent gelatin solution, the pH of which was previously adjusted at the desired value was buffered with 4 c.c. of McIlvaine's phosphate-citric acid buffer, 2 c.c. of 2.5 per cent enzyme solution was added and the mixture was digested in a glass-stoppered bottle at 45°C. for different periods. After every ten minutes, 5 c.c. of the digestion mixture was taken out and added to 45 c.c. of absolute alcohol which was previously heated to 50-60°C and the mixture was titrated with N/20 alcoholic KOH from a micro burette using thymolphthalein as an indicator. A blank titration was carried out in an identical manner using boiled enzyme solution. The proteolytic activity of the enzyme was expressed as the net titre value (ml. of N/20 KOH) per 5 c.c. digestion mixture.

In order to study whether the use of the buffer during proteolysis could be dispensed with, the above experiments were repeated without the use of any buffer. 4 c.c. glass-distilled water was used instead of 4 c.c. buffer solution. The results obtained are presented in Table I. As it was observed that there was no appreciable variation of pH of the digestion mixture within half an hour period of hydrolysis and as the data obtained with and without the use of buffer were practically of the same order, the use of buffer was dispensed with in all further experiments.

TABLE I

Estimation of proteolytic activity by alkali titration method.

Enzyme used	pH of substrate	Period of hydrolysis mins.	Proteolytic activity (ml. of N/20 KOH per 5cc digestion mixture) With buffer	Without buffer
Trypsin	8.0	10	1.80	1.81
		20	1.76	1.74
		30	1.81	1.81
Pepsin	2.2	10	1.38	1.37
		20	1.62	1.62
		30	1.57	1.58
Papain	5.0	10	2.01	2.02
		20	1.85	1.84
		30	1.90	1.92
Calotropain	4.2	10	2.10	2.10
		20	2.02	2.03
		30	2.24	2.23

II. FORMOL TITRATION METHOD :

The method which was first developed by Northrop [4] was modified in the present investigation. To 50 c.c. of 5 per cent gelatin solution of desired pH, 2 c.c. of 5 per cent enzyme solution was added and the mixture was digested at 45°C. for different periods. After every ten minutes, 10 c.c. of the digestion mixture was taken out, 2 c.c. of neutralised 40 per cent formaldehyde solution was added and the mixture was whirled for 5 minutes and titrated with N/10 NaOH solution from a microburette using phenolphthalein as an indicator. A blank titration was carried out in a similar manner using boiled enzyme solution. The proteolytic activity of the enzyme was expressed as the net titre value (ml. of N/10 NaOH) per 10 c.c. digestion mixture. The results obtained are given in Table II.

TABLE II

Estimation of proteolytic activity by formol titration method.

Enzyme used	pH of substrate	Proteolytic activity (ml. of N/10 NaOH per 10c c. digestion mixture)		
		10 mins. hydrolysis.	20 mins. hydrolysis.	30 mins. hydrolysis.
Trypsin	8.5	0.23	0.68	1.73
Pepsin	2.0	0.31	0.65	1.53
Papain	5.2	0.34	0.83	1.81
Calotropain	4.0	0.60	1.06	2.20

III. LOHLEIN—VOLHARD METHOD :

This method as adopted by Kuntzel[5] was followed by us with suitable modifications. To 25 c.c. of 5 per cent egg-albumin solution of desired pH, 1 c.c. of 5 per cent enzyme solution was added and the mixture was allowed to digest at 45°C for different periods. After every ten minutes, 5 c.c. of the digestion mixture was taken out, 5 c.c. of N/5 HCl was added to stop enzyme action and then 10 c.c. of 10 per cent sodium sulphate solution was added to precipitate undigested protein. After several minutes of cooling, the mixture was filtered and the filtrate was titrated with N/10 NaOH using phenolphthalein as an indicator. A blank titration was carried out in an identical manner using boiled enzyme solution. The proteolytic activity of the enzyme was expressed as the net titre value (ml. of N/10 NaOH) per 5 c.c. digestion mixture. The results obtained are presented in Table III.

TABLE III

Estimation of proteolytic activity by Lohlein-Volhard method.

Enzyme used	pH of substrate	Proteolytic activity (ml. of N/10 NaOH per 5 c. c. digestion mixture)		
		10 mins. hydrolysis.	20 mins. hydrolysis.	30 mins. hydrolysis.
Trypsin	8.3	0.13	0.24	0.52
Pepsin	2.2	0.11	0.18	0.34
Papain	6.0	0.20	0.36	0.65
Calotropain	4.2	0.27	0.40	0.89

IV. GROSS-FULD METHOD :

The method as modified by Chambard and Doubiran[6] was followed by us with suitable modifications. In a series of ten test tubes, increasing amounts (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 c.c.) of 0.1 per cent enzyme solution were taken, the volume in each tube was made upto 5 c.c. with glass-distilled water and 5 c.c. of 0.2 per cent gelatin solution of desired pH was added and mixed in each tube as rapidly as possible. The tubes were incubated at 45°C. for exactly half an hour and then 1 c.c. of the modified Esbach's solution[6] was added into each tube. The first tube in which there was no turbidity was noted with the help of a Klett Nephelometer. The proteolytic activity of the enzyme was expressed as Gross-Fuld unit which was equal to

$$\frac{\text{mgms of gelatin in first clear tube}}{\text{mgms of enzyme in the same tube}} \times 100.$$

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When turbidity appeared in each of the ten tubes, the experiments were repeated using greater amounts of the enzyme upto a maximum of 5 c.c. of 0.1 per cent enzyme solution. The above set of experiments were repeated on varying the period of hydrolysis to 10 and 20 minutes. The results obtained are presented in Table IV.

TABLE IV

Estimation of proteolytic activity by Gross-Fuld method.

Enzyme used	pH of substrate	Proteolytic activity (Gross-Flud unit)		
		10 mins. hydrolysis	20 mins. hydrolysis	30 mins. hydrolysis.
Trypsin	8.4	333	625	1250
Pepsin	2.2	278	500	1000
Papain	5.0	417	714	1667
Calotropain	4.0	500	1250	2500

V. GELATIN VISCOSITY METHOD.

The method which was developed by Northrop[4] was followed with suitable modifications. The Ostwald Viscometer of capillary type (B.S.S. No. 188) was used. The viscometer was clamped vertically in a glass-walled thermostatic bath set at 45°C ± 0.01 12.5 c.c. of 2 per cent gelatin solution of desired pH was pipetted into a test tube and was kept immersed in the bath till

it attained the temperature of the bath. 0.2 c.c. of 5 per cent enzyme solution was then added, mixed well and the time of addition noted. 10 c.c. of the mixture was poured into the wide arm of the viscometer. The liquid was drawn up into the bulb with gentle suction and the time of the outflow was measured with a stop-watch after hydrolysis for 30 minutes. A control experiment was carried out in an identical manner using boiled enzyme solution. The proteolytic activity of the enzyme was expressed as the percentage reduction in the time of the outflow of the experimental as compared to that of the control. The above set of experiments were repeated on varying the period of hydrolysis to 10 and 20 minutes. The results are presented in Table V.

TABLE V

Estimation of proteolytic activity by Gelatin-viscosity method.

Enzyme used	pH of substrate	Proteolytic activity (per cent reduction in the time of outflow)		
		10 mins. hydrolysis	20 mins. hydrolysis	30 mins. hydrolysis.
Trypsin	8.3	12.4	25.8	39.0
Pepsin	2.1	11.0	22.3	33.7
Papain	5.5	14.6	29.5	45.1
Calotropain	4.3	16.8	33.1	51.8

VI. NON-PROTEIN NITROGEN METHOD.

The method of Northrop [4] was followed with suitable modifications. To 25 c.c. of 5 per cent egg-albumin solution of desired pH, 1 c.c. of 5 per cent enzyme solution was added and the mixture was allowed to digest at 45°C. for different periods. After every ten minutes, 5 c.c. of the digestion mixture was pipetted into a centrifuge cup, 5 c.c. of 10 per cent trichloracetic acid was added and the mixture was warmed on a water-bath and centrifuged. The clear liquid was decanted out and analysed for total nitrogen by micro-Kjeldahl method. A control experiment was carried out in an identical manner using boiled enzyme solution. The results were expressed as mgms. of non-protein nitrogen per 5 c.c. digestion mixture. The results are presented in Table VI.

TABLE VI

Estimation of proteolytic activity by non-protein nitrogen method.

Enzyme used	pH of substrate	Proteolytic activity (mgs. of n-P. N. per 5 c. c. digestion mixture)		
		10 mins. hydrolysis	20 mins. hydrolysis	30 mins. hydrolysis.
Trypsin	8.5	5.7	12.4	18.0
Pepsin	2.2	5.0	10.6	16.1
Papain	5.0	6.5	12.7	19.6
Calotropain	4.2	6.8	15.0	23.4

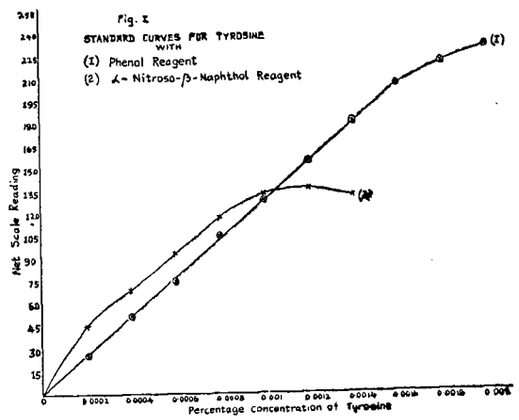
VII. COLORIMETRIC METHODS :

A. The colorimetric method of Anson [7] for estimating the liberated tyrosine during proteolysis was followed with suitable modifications making use of a photo-electric colorimeter.

Standard curve : 1.0 per cent. solution of pure tyrosine in 0.2 N HCl was prepared and serially diluted to form different strengths of the tyrosine solution ranging from 0.0002 to 0.002 per cent. 5 c.c. portions of these solutions were taken in 250 c.c. conical flasks, 10 c.c. of 0.5 N NaOH were added, mixed well and then 3 c.c. of phenol reagent which was diluted twice before use were added. The flasks were whirled for exactly two minutes and the intensity of the blue colour developed in each solution was measured in Klett-Summerson photo-electric colorimeter using different light filters. The red filter of 660 millimicrons gave the largest difference in scale reading between the lower and higher tyrosine concentration and was therefore selected for this study. A reagent blank was run in an identical way by using 5 c.c. distilled water instead of 5 c.c. tyrosine solution and the reading was subtracted from the previous readings. The net scale readings were plotted on a graph paper against the concentration of the tyrosine solutions used and that portion of the curve which gave a straight line obeying Beer's Law was selected as the standard curve for all further reference. The standard curve is shown in Fig. I.

In order to determine the amount of tyrosine liberated during proteolysis, 1 c.c. of 5 per cent. enzyme solution was added to 25 c.c. of 5 per cent. egg-albumin solution of desired pH and the

mixture was allowed to digest at 45°C for different periods. After every 10 minutes, 5 c.c. of the digestion mixture was then pipetted into a centrifuge cup, 10 c.c. of 0.3 N trichloracetic acid



was added to precipitate the undigested protein. The mixture was warmed on the water-bath and centrifuged. The clear centrifugate was suitably diluted so that the net scale reading after subtraction of the reading for the control might fall within the range of the standard curve. To 5 c.c. of the diluted solution, 10 c.c. of 0.5 N NaOH and 3 c.c. of diluted phenol reagent were added and mixed up for two minutes as before and the colour developed was read in the photo-electric colorimeter with red filter No. 66. A control experiment was run in an identical manner using boiled enzyme solution. The proteolytic activity was expressed as milligrams of tyrosine liberated from 5 c.c. digestion mixture. The results obtained are presented in Table VII.

B. Since the basic principle of Anson's colorimetric method [7] for estimating proteolytic activity is the estimation of liberated tyrosine during proteolysis, it was thought desirable to try other colour reactions of tyrosine. It was reported [8] that α-nitroso-β-naphthol was a specific colour reagent for tyrosine. Taking advantage of this specificity, Thomas[9] developed a photelometric method for the determination of tyrosine in proteins. Making use of a photo-electric colorimeter, this method was tried with suitable modifications for the estimation of the liberated tyrosine during enzymic hydrolysis of proteins.

Standard curve : A series of pure tyrosine solutions of different strengths ranging from 0.0002 to 0.0014 per cent. were prepared. 5 c.c. portions of these solutions were taken in test tubes and 1 c.c. of 0.12 per cent. α-nitroso-β-naphthol solution in 95% alcohol was added to each tube. 2 c.c. of concentrated HCl and 1 c.c. of nitric acid solution which was prepared by diluting 12 c.c. of conc. HNO_3 to 100 c.c. were then added. The contents of each tube were well mixed and the tubes were shaken in a boiling water-bath for exactly 47 seconds. They were then quickly cooled under running tap-water while shaking for exactly one minute. The colour developed in each solution was measured in Klett-Summerson photo-electric colorimeter with the green filter No. 54. A reagent blank was run in an identical manner by using 5 c.c. distilled water instead of 5 c.c. tyrosine solution. A sample blank was run by using 5 c.c. tyrosine solution, 1 c.c. 95% alcohol, 2 c.c. conc. HCl and 1 c.c. diluted HNO_3 solution as before. The readings on these blanks were subtracted from the previous readings. The net scale readings were plotted on a graph paper against the concentration of the tyrosine solutions used. Only a small portion of the curve was found to give a straight line obeying Beer's Law. The standard curve is presented in Fig. I.

In determining the amount of tyrosine liberated during proteolysis, 1 c.c. of 5 per cent. enzyme solution was added to 25 c.c. of 5 per cent. egg-albumin solution of desired pH and the mixture was allowed to digest at 45°C for different periods. After every ten minutes, 5 c.c. of the digestion mixture was then treated with 10 c.c. of 0.3 N trichloracetic acid, warmed and centrifuged. The clear centrifugate was suitably diluted so that the net sale reading after subtraction of the reading for the control might fall within the range of the standard curve. To 5 c.c. of the diluted solution, 1 c.c. of 0.12 per cent. α nitroso-β naphthol solution in 95% alcohol, 2 c.c. of conc. HCl and 1 c.c. of diluted HNO_3 solution were added and mixed up as before. The tubes were shaken in a boiling water-bath for 47 seconds, cooled with shaking under tap water for one minute and the colour developed was read in the photo-electric colorimeter with the green filter No. 54. A control experiment was run in an identical manner using boiled enzyme solution. The amount of tyrosine liberated was calculated with reference to the standard curve. The proteolytic activity was expressed as milligrams of tyrosine liberated per 5 c.c. digestion mixture. The results obtained are presented in Table VII.

C. It is well known that tyrosine and tryptophane give positive Millon's reaction. Taking advantage of this reaction, Lugg [10] developed a colorimetric method for the quantitative estimation of tyrosine and tryptophane in proteins. Utilising the basic prin-

ciples of this method and making use of a photo-electric colorimeter, a new method was developed for the quantitative estimation of tyrosine liberated in enzymic hydrolysis of proteins.

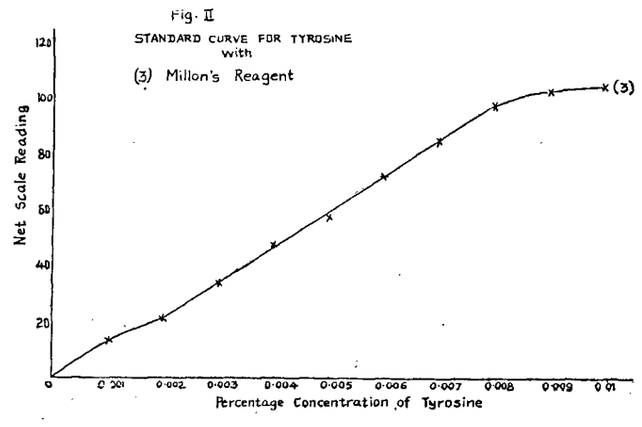
REAGENT SOLUTIONS

Lugg's Reagent[10] : 75 gms $HgSO_4$, 55 gms. $HgCl_2$ and 70 gms. Na_2SO_4 were dissolved in 850 c.c. water and 125 gm. 98% H_2SO_4 and diluted to one litre.

Millon's Reagent was prepared as per the standard procedure of digesting one part by weight of mercury (A.R.) with two parts by weight of conc. HNO_3 and after complete digestion, adding two volumes of water.

Standard curve : A series of pure tyrosine solutions of different strengths ranging from 0.001 to 0.01 per cent were prepared. 3 c.c. portions of these solutions were taken in centrifuge tubes, 2 c.c. of 5 N H_2SO_4 and then 5 c.c. of Lugg's reagent were added and the tubes were heated at 60-65°C in a water-bath for exactly 30 minutes after which they are cooled in running tap water for one hour. 5 c.c. portions of these solutions were taken in 100 c.c. conical flasks, 0.5 c.c. of the Millon's reagent were added and the flasks were whirled for exactly 3 minutes and the colour developed in each solution was measured in Klett-Summerson photoelectric colorimeter using green filter No. 54. A reagent blank was run in an identical manner by using 3 c.c. distilled water instead of 3 c.c. tyrosine solution and the reading was subtracted from the

Fig. II



previous readings. The standard curve was drawn by plotting the net scale readings against the concentration of the tyrosine solutions as before. The standard curve is presented in Fig. II.

In order to determine the amount of tyrosine liberated during enzymic hydrolysis of proteins, 1 c.c. of 5 per cent enzyme solution and 25 c.c. of 5 per cent egg-albumin solution of desired pH were mixed well and allowed to digest at 45°C for different periods. After every ten minutes, 5 c.c. of the digestion mixture was then treated with 10 c.c. of 0.3 N trichloracetic acid, warmed and centrifuged. 3 c.c. of the clear centrifugate was taken in a centrifuge tube, 2 c.c. of 5 N H_2SO_4 and then 5 c.c. of the Lugg's reagent were added to precipitate any tryptophane. The tube was heated at 60-65°C. in a waterbath for exactly 30 minutes after which it was cooled under running tap-water for one hour and then centrifuged for 5 minutes. The clear centrifugate was diluted, if necessary, and to 5 c.c. of this solution, 0.5 c.c. of the Millon's reagent was added, the flask was whirled for exactly 3 minutes and the colour developed was measured in the photoelectric colorimeter with green filter No. 54, as before. A control experiment was run in an identical manner using boiled enzyme solution. The amount of tyrosine liberated was calculated with reference to the standard curve. The proteolytic activity was expressed as milligrams of tyrosine liberated per 5 c.c. digestion mixture. The results are presented in Table VII.

TABLE VII.

Estimation of protolytic activity by different colorimetric methods.

Enzyme used	pH of substrate	Period of hydrolysis minutes	(Proteolytic activity (mgms. tyrosine liberated per 5 c c. digestion mixture)		
			Phenol reagent	α-nitroso-β naphthol reagent	Millon's reagent
Trypsin	8.4	10	0.47	0.35	—
		20	0.95	0.73	—
		30	1.44	1.10	1.15
Pepsin	2.2	10	0.39	0.32	—
		20	0.78	0.61	—
		30	1.18	0.90	1.00
Papain	5.5	10	0.54	0.40	—
		20	1.10	0.82	—
		30	1.62	1.22	1.30
Calotropain	4.5	10	0.62	0.52	—
		20	1.22	1.01	1.00
		30	1.90	1.53	1.58

In order to test the accuracy of the colorimetric methods in estimating very low concentrations of tyrosine, the following experiment was carried out. In a series of five centrifuge tubes, increasing amounts (0.05, 0.1, 0.5, 1.0, 1.2 c.c.) of 0.1 per cent pure tyrosine solution were taken, the volume in each tube was made up to 5 c.c. by adding required amounts of 5% egg-albumin solution. Then 10 c.c. of 0.3 N trichloracetic acid were added in each tube, the mixture was warmed on the water-bath and centrifuged. The clear centrifugate was analysed for the tyrosine content by the different colorimetric methods as described before. With respect to each method, a control experiment was carried out in an identical manner using distilled water instead of tyrosine solution and the value was subtracted from the previous values. The results obtained are presented in Table VIII.

TABLE VIII

Recovery of known amounts of tyrosine by different colorimetric methods.

Tyrosine added mgms.	Tyrosine found, mgms.			Recovery Percent		
	Phenol reagent	α-nitroso-β-naphthol reagent	Millon's reagent	Phenol reagent	α-nitroso-β-naphthol reagent	Millon's reagent
0.05	0.049	—	—	98	—	—
0.10	0.098	0.097	—	98	97	—
0.50	0.49	0.49	—	98	98	—
1.00	0.99	0.97	1.00	99	97	100
1.20	1.18	1.18	1.19	98.3	98.3	99.2

DISCUSSION

As described under experimental, suitable modifications were introduced in each method tried. All the modified methods except the alkali-titration method were found to give more or less reproducible data. In the case of the alkali titration method, it was noted that the end point of the titration was not very sharp and because of the difficulty in detecting the end point correctly, uniform results were not obtained.

although the modified Gross-Fuld method in which the Klett Nephelometer was used for the detection of turbidity of the test solution and the modified formol titration and Lohlein-Volhard methods were found to give quite reproducible results, these

methods were not sufficiently sensitive to measure accurately the small difference in the degree of hydrolysis of the protein, which occurred with varying periods of hydrolysis. Hence, these methods were not very efficient for accurate quantitative measurement of the proteolytic activity after short period of hydrolysis. The modified formol titration method was quite simpler and less time-consuming. However, as this method was related with the measurement of the increase in the titratable carboxyl groups which is only a very small per cent of the total titration of the test solution, the titration had to be carried out with utmost care.

Although the viscosity reduction method was found to be quite simple and rapid and capable of recording even mild proteolytic activity, the method was applicable only under limited conditions. The use of gelatin as a substrate was essential in this method. Although casein-viscosity method was also reported [11] for proteinase evaluation, gelatin is more commonly used as a substrate in the viscosity reduction method in view of the advantages that gelatin easily forms homogeneous solution and also remains in solution over the entire pH range. The use of a suitable concentration of the substrate is also very essential in this method. It therefore appears that this method may not be satisfactorily applied for studying the effect of an enzyme on different substrates or on different concentrations of the same substrate.

The non-protein nitrogen method was found to give quite uniform and reproducible results. In the case of this method, however, such of the proteins which are not quantitatively precipitated by trichloracetic acid cannot be used as substrates. For this reason, gelatin cannot be used as a substrate in this method.

Of all the methods tried, the modified colorimetric method making use of the phenol reagent and the photo-electric colorimeter with the red filter No. 66 was found to be the best method for routine quantitative estimation of proteolytic activity. The method was found to give very accurate and reproducible data. The results given in Table VIII show that the method was capable of recovering 98 to 99 per cent of the known amounts of tyrosine. As the straight line portion of the standard curve (Fig. I) for tyrosine with phenol reagent provided a wide range from 0 to 0.0016 per cent concentration of tyrosine solution, the method was capable of estimating accurately even very low contents of tyrosine and hence very small proteolytic activity.

The colorimetric method making use of α nitroso β naphthol as a colour reagent was also found to give quite accurate and reproducible results, the recoveries of known amounts of tyrosine being 97 to 98 per cent (Table VIII). In this case, however, as the straight line portion of the standard curve provided only a very small range from 0.0004 to 0.0008 per cent. concentration of tyrosine solution, the dilution of the test solution had to be done with great care and also estimations of tyrosine below 0.0004 per cent. concentration were not possible by this method. Hence this method was not suitable for routine quantitative assays of proteolytic activity.

In the case of the colorimetric method with Millon's reagent, it was observed that the method was capable of giving accurate results only when the concentration of tyrosine in test solution was above 0.002 per cent., as the standard curve (Fig. II) for tyrosine with Millon's reagent gave a straight line portion only within the range of tyrosine concentration of 0.002 to 0.008 per cent. It is, therefore, obvious that by this method accurate estimations of very mild proteolytic activity were not possible.

Obviously, all the three colorimetric methods are applicable for studying the proteolytic activity against such of the proteins which contain reasonable amount of tyrosine and which are precipitated by trichloracetic acid. Although the phenol reagent develops blue colour with both tyrosine and tryptophane and although the total colour produced by both the amino-acids is measured by this method, for the sake of reproducibility Anson [7] expressed the results in terms of tyrosine only. The phenol reagent method is, therefore, applicable for recording comparative proteolytic activity only when the same protein is used as a substrate throughout the investigation and is not suitable for comparative studies against different proteins containing varying proportions of tyrosine and tryptophane. The α-nitroso β naphthol method, however, is free from this drawback, as this reagent is specific for colour reaction with tyrosine only. As the Millon's reagent also develops colour with both tyrosine and tryptophane, in the case of the Millon's reagent method, tryptophane was removed from the enzymic hydrolysate of the protein by Lugg's method [10] and the colour reaction was allowed to take place with tyrosine only. The α nitroso β naphthol method and the Millon's reagent method which have been developed in the present investigation are therefore applicable for estimation of comparative proteolytic activity against different proteins containing both tyrosine and tryptophane.

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REFERENCES

1. Bose, S. M. and Madhava Krishna, W., *Enzymologia*, under publication.
2. Willstatter, R. and Waldschmidt-Leitz, *E. Ber.*, 1921, **54**, 2988.
3. Macrae, T. F., *Biochem. Jour.*, 1933, **27**, 1229.
4. Northrop, J. H., *Jour. Gen. Physiol.*, 1932, **16**, 41.
5. Kuntzel, A., *Collegium*, 1932, 409.
6. Chambard, P. and Doubiran, M., *Jour. Soc. Lea. Trad. Chem.*, 1938, **22**, 333.
7. Anson, M. L., *Journ. Gen. Physiol.*, 1938, **22**, 79.
8. Gerngross, O., Voss. K., and Herfeld, H., *Ber.*, 1933, **66B**, 435.
9. Thomas, L. E., *Arch. Biochem. & Biophys.*, 1944, **5**, 175.
10. Lugg, J. W. H., *Biochem. Jour.*, 1937, **31**, 1422.
11. Nandy, S., and Banerjee, R. P., *Jour. Lea. Tech. Assoc. (India)*, 1955, **3**, 37.

RESEARCH BEARS FRUIT

Practical Demonstration No. 3 Process No. 1

MANUFACTURE OF PICKING BAND LEATHER BY THE COMBINATION OF SULPHUR-OIL-VEGETABLE TANNING METHOD

S. N. BOSE

Raw material : 60 to 65 lbs w/s prime quality buff. hides without flay-cuts or grain damages like scratches or warble holes etc., are desirable.

Soaking : The hides are soaked overnight for about 18 hours in the following bath :

Caustic soda	..	$\frac{1}{4}$% on wet-salted weight.
Common salt	..	7% „
Water	..	500% „

Bleaching powder — $\frac{3}{4}$ lbs. per 500 lbs. of water.

Next day, the hides are washed in two changes of plain water and then they are rounded into butt, belly and shoulder etc.

The butts are taken for picking band manufacture whereas the belly, shoulder, etc., may be processed into picker or lace or sole.

The butts are fleshed to remove any big patch of flesh if there be any, and after washing again in two changes of water, they are limed.

Liming : 250% to 300% water is taken and to it 5% slaked lime is added. Butts are agitated in it for 10 mts. after which they are hauled up and 1% sodium sulphide dissolved in minimum quantity of water and cooled is added to the bath. The butts are again handled in it for 10 mts. and then they are left in the liquor for two hours after which the butts are hauled up and replaced. This is repeated once again in the evening after which they are kept immersed in the liquor overnight.

Next morning, the butts are hauled up and 1% sodium sulphide and 5% slaked lime are added to the bath and mixed well. Then the butts are agitated in it and left therein for 2 hours after

which hauling and replacing are done. This is repeated once again in the evening whereafter they are kept immersed in the liquor overnight. On the third day, the butts are hauled up and replaced three times at an interval of 2 hours. In the evening, the butts are kept immersed in liquor overnight. On the fourth day, the butts are unhaired, washed, splitted if necessary to about 8 mm. and then they are fleshed, washed again and scudded and weighed. The scudded butts are washed in two changes of water and then delimed. All the chemicals are calculated on the weight of the limed pelt.

Deliming : Ammonium sulphate — 3% on pelt weight.
Water — 150% on pelt weight.

The butts are put in the drum (speed of drum 10 r.p.m.) containing the above materials and the drum is run for one hour and kept stopped for one hour, and again the drum is run for one hour after which the butts are kept immersed in the bath overnight. Next day, the drum with the goods and liquor is run for one hour or a little more whereafter the cut edge showing neutral colour with phenolphthalein, the butts are taken out, scudded if desired and washed in two changes of water and then they are pickled.

Pickling : Sulphuric acid (66° Be) — $2\frac{1}{2}$ to 3% on pelt wt.
Common salt — 10 to 12% on pelt wt.
Water — 125 to 150% on pelt wt.

5 to 6% of common salt is dissolved in 100 to 125% of water. 1 to $1\frac{1}{4}$% acid is added to the salt solution and drummed for 10 mts. whereafter the butts are put in and drummed for one hour and kept stopped for the following one hour. In the meantime, the remaining $1\frac{1}{2}$ to $1\frac{3}{4}$% acid is diluted with 5% to 10% water and 5 to 6% salt in 20 to 15% water. After rest period of one hour, the drum is started and the salt and acid solutions after mixing is added to the drum slowly and the drum is run for one hour whereafter again stopped for one hour. Subsequently the drum may be run for 15 mts. every hour and kept stopped for remaining period. In the evening the goods are kept immersed in the bath overnight.

Next day, the drum is alternately run for $\frac{1}{2}$ hour and given rest for $\frac{1}{2}$ hour over a period of 5 to 6 hours. At this stage the cut edge should show red colour with methyl red. When this test is satisfactory, the goods are taken up and horsed up for 24 hours. The speed of drum for pickling and depickling is 10 r.p.m.

Depickling : Hypo — 15 to 20% on pelt weight.
Water — 60 to 80% on pelt weight.

The hypo is dissolved by drumming in the water. The goods are put in and the drum run for one hour, stopped for one hour and thereafter for the subsequent period drumming for ½ hour and stopping the drum for ½ hour may be repeated till the evening when the goods are immersed in liquor as far as possible.

Next day, the drum is run for one hour, stopped for one hour, and thereafter alternately run for ½ hour and stopped for ½ hour for over a period of 6 hours when the freshly cut edge shows yellowish colour with methyl Red indicator solution. The butts are horsed up for one day at least or for even two days. Then the butts are hung up overnight for sammying. The sammed butts are dry drummed for ½ hour, then shaved to remove any fleshy material that may be still adhering and stuffed as follows :

Stuffing :

1st stuffing : Mutton tallow — 4½ to 5% on pelt wt.
Fish oil — 4½ to 5% on pelt wt.

Temperature of the stuffing drum (15-18 r.p.m.) is raised to 40°C. Tallow is melted by heating and to it is added fish oil. The temperature of the mixture may be brought to 45°C and the heated mixture of tallow and fish oil may be added to the drum. The shaved butts are fed in the drum and the drum is run for two hours whereafter the butts may be hung up in a hot room of temperature 40°C for about a week whereafter the second stuffing may be given using the same percentage of tallow and fish oil and following the same procedure. The butts may be hung up in hot room for oxidation for about 8 to 10 days and when oxidation is considered to be over which is tested by cutting a thick piece whose cut section should be yellow throughout, by the soft feel of the butt and by the presence of oxidised smell and formation of acraldehyde and by the absence of the predominance of the smell of either fish oil or tallow. The well stuffed butts may be kept under stretch for one day whereafter the butts may be slicked out for removing any surface fat, etc., especially from flesh side or the latter may be shaved a little to remove the surface excess fat if there be any.

Vegetable tanning :

A 75° Bk. sulphited quebracho extract liquor is prepared. 100% float of this liquor on pelt weight is taken in drum to which

is added 0.5% T.R.O. and 0.15% Paranitrophenol dissolved in a little warm water. (Both T.R.O. and paranitrophenol are taken on pelt weight). The drum is set in motion and the butts which have been dry drummed for ½ hour are put in and drummed for 2 hours, and thereafter alternately stopped for ½ hour and run for ½ hour, over a period of six hours till the evening when the butts are kept immersed in liquor overnight.

Next day, the drum is again likewise run and stopped alternately for 6–8 hours whereafter the butts are taken out and left overnight in sufficient float of water to remove the loose tannin.

Next morning, the butts are scoured, washed, slicked and oiled up with either a mixture of one part pungam oil and one part glycerine or two parts mutton tallow and one part pungam oil, hung up for sammying, set out well and hung up for one day for drying. Taken out and cut into required size.

Yield—55% on pelt weight.

TECHNICAL SEMINAR *

Fundamentals of X-ray diffraction and some application to the Collegen System

K. S. CHANDRASEKARAN.

SUMMARY

The speaker started with a brief review of some of the fundamentals of the diffraction of X-rays by crystals. The different methods of obtaining the diffraction photographs are discussed. The experimental data available from such pictures is of two types : one, the positions of the diffraction spots and the other, their intensities. The former information relates to the determination of the until cell, molecular weight and space group while the intensity data is the starting point for complete structure analysis. A few applications of such techniques of interest to chemists were briefly pointed out.

The ' oriented crystallisation ' of some inorganic salt solutions into the collagen was then discussed as an example of X-ray study of collagen where the diffraction photograhps directly showed the effect. The work of Mr. G. K. Ambady at the physics department of the University of Madras was explained and it was shown that the results of this work was in favour of the structure of collagen proposed from that laboratory.

* Summary of the Seminar held on 22-3-1957.

Shri C. Subramaniam, Minister for Finance, Government of Madras, presiding over the inauguration of the symposium on 28th March, 1957. Dr. Y. Nayudamma, Assistant Director-in-charge, seated to his right.

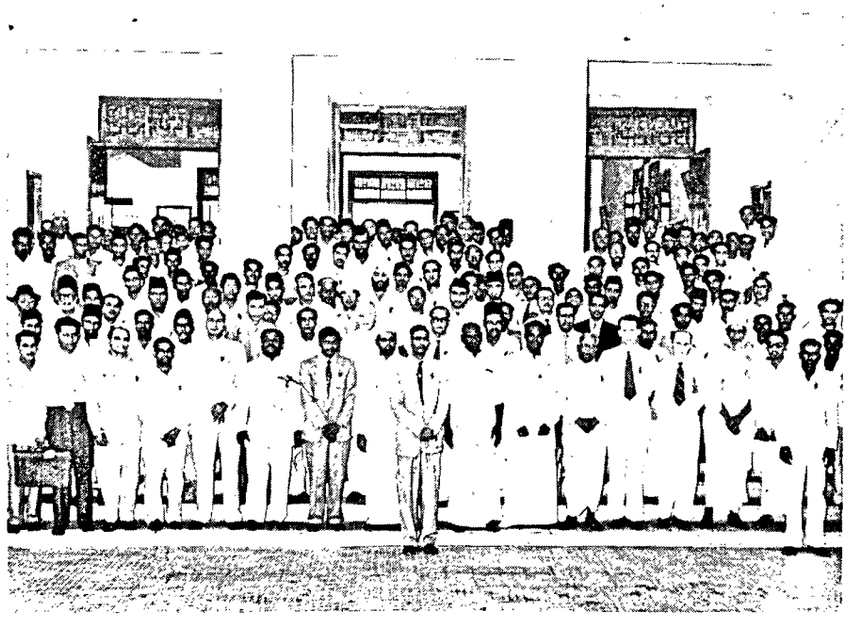

Photo of the delegates to the symposium with Hon'ble Shri K Kamaraj, Chief Minister, Government of Madras.

"AT HOME" TO DELEGATES

Left to Right :
Dr. L. M. Hira, Mr. N. M. Anwar, *Chief Guest* Shri K. Kamaraj, Chief Minister, Govt. of Madras, Dr. Y. Nayudamma, Mr. A. Nagappa Chettiar, Mr. Duraivelu and Mr. G. S. Srinivasa Iyer.

Dr. Y. Nayudamma explaining the staking operation to H.E. Shri A. J. John and Mrs. A. J. John when they visited the institute.

SYMPOSIUM ON RAW HIDES AND SKINS—CURING AND PRESERVATION

The three-day symposium on Raw Hides and Skins — Curing and Preservation was inaugurated by Prof. M. S. Thacker, Director-General, Scientific & Industrial Research, New Delhi, at the Central Leather Research Institute on Thursday, the 28th March 1957 at 3 p.m. before a distinguished gathering of hides and skins merchants and other officials connected with the development of leather industry. Hon'ble Shri C. Subramaniam, Minister for Finance and Education, Government of Madras, presided.

Dr. Y. Nayudamma, Assistant Director-in-charge of the Institute, welcomed the Finance Minister and the delegates who had come from the various parts of the country and said that the industry had been receiving much help from the Finance Minister and they were confident that Mr. Subramaniam would continue to be the Finance Minister in the new Cabinet also and extend to the industry the same kind treatment as hitherto. He explained the value of the symposium on an important subject like "Raw Hides and Skins—Curing and Preservation" and expressed the hope that the conference would be able to formulate schemes for the development of the industry. They were all sorry, he said, that Prof. Thacker could not be present on the occasion owing to urgent duties in Delhi.

Mr. N. S. Mani read the messages from H. E. Sri A. J. John, Governor of Madras, Director, Australian Leather Research Association, Sydney, President, Society of Leather Trades Chemists, London, Associated Chamber of Hide and Skin Traders, Calcutta, Mr. Valenta of Messrs. Bata Shoe Co. (Private) Ltd., Batanagar, Bengal Tanning Institute, Calcutta, The Kathiawar Industries Ltd., Skins & Leathers Private Ltd., Coimbatore, The Editor, Tanner, Madras Chamber of Commerce, etc.

Mr. N. M. Anwar, Honorary Secretary, Southern India Skin and Hide Merchants Association, said that the hides and skins industry was an important one and goods worth about Rs. 250 millions were passing through Madras Port annually to other countries. It was not enough to export hides and skins. They should be able to export finished products also and earn more

money. For this, technical knowledge was necessary and, in that respect, they were all glad that the Central Leather Research Institute was doing valuable service under the guidance of Dr. Y. Nayudamma. They were all sorry for the death of Prof. Das who had done the pioneering work for the leather industry.

Mr. G. S. Srinivasa Aiyar, Vice-President, Southern India Hides and Skins Association, said that the defects that were generally seen in hides were those caused by failure to wash them as soon as they were flayed and to salt them. Facilities should be provided in the rural parts for properly salting the hides.

Prof. Thacker said in his inaugural address that he had been looking forward eagerly for the opportunity of meeting the representatives of tanning industry so that he might tell them how deeply they in the Council of Scientific and Industrial Research appreciated their very keen interest in the activities of the Central Leather Research Institute and the continued guidance and goodwill offered by them to that Institute. Referring to the symposium, Prof. Thacker said that a meeting of people of varied interests coming together for a good cause, namely the development of the Indian Leather Industry, was indeed a welcome feature and his pleasure was, therefore, all the greater in inaugurating the deliberations of that important symposium on "Raw Hides and Skins—Curing and Preservation". "India" he pointed out, "is singularly fortunate in the field of leather, being the largest single holder of livestock in the world. It is estimated that the leather industry fetches about 300 million rupees annually to the Indian Union.

Prof. Thacker said :

"Leather has been used from time immemorial and the uses and the market for it are ever expanding. With the introduction of the ban on cow slaughter in the majority of the States in India, a serious problem has arisen for the tanner. The quality of the raw hides and skins is reported to be deteriorating gradually and the tanner and the tanning technologist are faced with the challenge to produce good quality leather from bad hide. An expanding market for leather and the increasing hide deterioration militate against each other and this basic fact calls for a searching of minds, pooling of thoughts, sharing of ideas and chalking out of a clear-cut co-ordinated programme. Realising the importance of this problem, the Governing Body of the Council of Scientific and Industrial Research approved of the holding of this symposium so

that the various sections of the tanning industry, namely, the livestock man, the flayer, the raw hide dealer, curer, distributor, the tanner, the finisher and the seller could be brought together to discuss the problems. The tanner is mainly interested in raw hides and skins. The prosperity of this industry, therefore, depends to a very large extent on how far you are willing to join together in a concentrated effort and do everything in your power to develop the quality of hides and skins and to meet the increasing demand for finished leather."

Explaining the purpose of the symposium, Prof. Thacker said : "I sincerely trust that this symposium organised by the Central Leather Research Institute and this coming together of all the segments of this industry having a vital interest in raw hides and skins will pave the way to find proper solutions for your problems. The Council of Scientific and Industrial Research has established 17 National Laboratories all over India. Each one of them is dedicated to the service of the industry to which it is allied. It is the desire of the Council and that of the Central Leather Research Institute should serve as a bridge between science and industry. Most of you believe in investigating your own methods of doing business, your accounting, financial structure, your sales and sales promotion—all of which you do with the singular idea of improving the figures of your balance sheet. I am sure that most of you realise that such investigations on the business side are only one aspect of the matter. There is still another aspect. This investigation is closely related to the figures in your balance sheet and this goes by the name of "Research". It is to conduct this research and to disseminate the results of such research to the practical tanner, that the Central Leather Research Institute has been established.

Mr. Subramaniam said that he was sure that exchange of ideas between the delegates from all over India would benefit the leather industry and he had no doubt that they would be able to make very good progress in utilising those resources to the best advantage of the nation. If the industry was to properly develop, they should get first-rate hides and skins. In that context, the attention that the Government were paying to the development of animal husbandry was helpful to the industry though it might be in an indirect way. He said he had occasion to visit some tanneries in the course of his election campaign and he found that very primitive methods were still being used for slaughter and flaying of animals.

The Minister said that from what he could see from the recent cattle show he was of the view that they were making good progress in the development of the cattle wealth. That would help them to get better hides and skins. The necessity for proper curing and preservation was emphasised by the previous speakers and they pleaded for facilities for the tanneries in the State. He said that he agreed that tannery was an important cottage industry and it should be helped. The difficulty was they were all dispersed over the State in small units. The need for proper method of flaying was emphasised. To meet that demand, the Government had started training course in flaying in the Veterinary College. The complaint was that those trained in the course were not able to find employment. That was a serious problem and so he would plead with the representatives of the trade to help them as, otherwise, the training course would not attract men. He suggested that the best way to get well flayed and salted hides and skins from the rural parts would be for the butchers there to form co-operatives. The representatives of the trade also could help them by giving them financial and other forms of aid.

Mr. Subramaniam assured that the Government would be always willing to help the industry, if proper schemes were formulated by the representatives of the industry and presented to them. The Government were quite aware that the industry was earning foreign exchange including dollars. For the development of the country, they had been depending upon foreign help to some extent and in return for that help they in this country should be able to give something. In that respect, the leather industry offered good scope. They should make proper and maximum use of the skins and hides available to them and in that respect, the research work done in the Central Leather Research Institute would be valuable. Dr. S. K. Barat, Senior Scientific Officer, Central Leather Research Institute, proposed a vote of thanks.

TECHNICAL SESSION I.

The first technical session of the symposium on "Raw Hides and Skins—Curing and Preservation" was held under the Chairmanship of Mr. R. K. Rangan, Joint Chief Controller of Imports and Exports, Madras and Ex-officio Chairman of the Leather Export Promotion Council, Government of India.

In his opening remarks, Mr. Rangan said that the main objects of the Leather Export Promotion Council were to promote exports of tanned hides and skins, to ascertain the requirements of buyers

with reference to quality and quantity and to assist the exporters to satisfy the requirements of the buyers. He hoped that ere long they would be able to satisfy the demands of the buyers and meet their requirements fully with the technical assistance available. For that, the co-operation of one and all connected with the industry was necessary. He said he was sure that the Government of India and all the State Governments would do all in their power to canalise and regulate the trade to the best advantage of the country.

The following papers were then presented :—

1. Future development of Indian Tanning Industry—By Mr. J. J. Walters, Guildford, Surrey, England.
2. Collection of raw goatskins on co-operative basis—By Shri R. V. Sovani, Manager, Dewas Tannery, Dewas.
3. Note on collection of raw hides and skins and suggestions for improved methods—By Dr. L. M. Hira, Superintendent of Markets, Greater Bombay.
4. Supply of quality raw hides and skins to Tanners' Co-operative Societies etc.—By C. V. Gangal, Tanning and Leather Expert, Industrial Co-operative and Village Industries, Poona.
5. Improvement of raw hides and skins—By N. S. Mani, Central Leather Research Institute, Madras.
6. Export of goatskins—By Calcutta Hide and Skin Shippers Association.

There was a discussion after all these papers were read and the Chairman winding up the discussion said that such symposia afforded an excellent opportunity for exchanging views on various points. He said that he was of the opinion that they got only a negligible return from the vast resources available in India because of lack of proper curing and preservation of the hides. He assured that the valuable suggestions made by the various delegates would be taken note of both by the Central Leather Research Institute and the Leather Export Promotion Council and would be closely examined by them in their respective spheres and all possible steps would be taken to find a solution to the problems confronting the industry. He requested the various delegates to communicate to them the results of the experiments conducted in their units

so that everybody might be benefited. He had no doubt whatsoever that with the co-operation of one and all, they could do a lot to improve the industry.

Mr. M. A. Ghani, Senior Scientific Officer, Central Leather Research Institute, proposed a hearty vote of thanks.

TECHNICAL SESSION II.

29th March 1957. (Forenoon).

The Second Session of the symposium was presided over by Dr. S. N. Sen, Senior Scientific Officer, Central Leather Research Institute, Madras, and the following papers were presented :—

1. Brining of animal hides—its merits and demerits—By Shri S. C. Nandy, Central Leather Research Institute, Madras.
2. Preservation of Indian goatskins for export—By Dr. M. Dempsey and Dr. B. M. Haines, British Leather Manufacturers' Research Association, Milton Park, Egham, Surrey.
3. Raw Hides and skins—Curing and Preservation—By Mr. D. Woodroffe, Northampton College of Technology, Northampton.
4. Factors affecting the salting of raw hides and skins and their preservation—By S. C. Nandy and S. N. Sen, Central Leather Research Institute.
5. Some aspects of curing practices—from Ministry of Commerce and Consumer Industries, Small Industries Service Institute.
6. Curing of hides and skins—By M. K. Bhagwat, State Industrial Co-operative Association Ltd., 9 Bake House Lane, Bombay-1.
7. Some considerations on the utilisation of bittern salt in curing—By S. C. Nandy and S. N. Sen, Central Leather Research Institute, Madras.
8. Pickling hides and skins as a method of preservation—By Shri M. K. Bhagwat, State Industrial Co-operative Association Ltd., Bombay.

9. Preservative efficiency of common salt in admixture with sodium pentachlorophenate—By S. C. Nandy and S. N. Sen, Central Leather Research Institute, Madras.
10. Discussion of curing and preservation—From M/s. Bata Shoe Co., Ltd., Batanagar.

There was a discussion on the subject after all the papers were presented. Dr. Sen in his concluding remarks said that it was necessary to have a Central Board of Curing and Storage so that advice might be sought from them if needed. That would stop the colossal waste which was sustained due to bad storage and bad curing.

Shri K. T. Sarkar, Senior Scientific Officer, Central Leather Research Institute, Madras proposed a vote thanks.

TECHNICAL SESSION III.

29th March 1957. (Afternoon).

Mr. B. N. Soni, Research Officer, Indian Veterinary Research Institute, Izatnagar, presided over this session. In his introductory remarks, Mr. Soni said that in dealing with insects, they had to deal with living animals and also biological factors. He said he had done plenty of research in the Indian Veterinary Research Institute and said he would explain the salient features while reading his paper.

The following papers were then presented :—

1. Some aspects of the biochemistry of raw hides and skins—By S. M. Bose, Central Leather Research Institute, Madras.
2. Studies on the prevention of insect damage to hides and skins—By R. Bhaskaran, Central Leather Research Institute, Madras.
3. Histological study of the effect of postmortem changes on the leather forming qualities of calf skin—By S. K. Sarkar and S. K. Mitra, Central Leather Research Institute, Madras.
4. A note on the damage caused to the skin of cattle, sheep, buffaloes and goats by parasites—By V. S. Alwar, Lecturer, Madras Veterinary College, Madras.

5. Defects in raw hides and skins and suggestions for improvement—By Director of Animal Husbandry, Andhra Pradesh.

6. Parasitic skin diseases of animals and their effects—By R. Bhaskaran, Central Leather Research Institute, Madras.

7. Viral, Bacterial and other diseases causing damage to live hides and skins—By K. P. Chandrasekaran and B. Narasinga Rao, Madras Veterinary College, Madras.

8. Certain parasitic infections of cattle involving the skin—By M. Anantaraman, Research Officer, (Helminthology), Madras Veterinary College, Madras.

9. Some defects in raw hides and skins and suggestions for their improvement—By Mr. B. N. Soni, Research Officer, Indian Veterinary Research Institute, Izatnagar (U.P.).

10. Impact of animal diseases on leather manufacture—By R. Bhaskaran, Central Leather Research Institute, Madras.

Then by the kind courtesy of Mr. F. H. Hoek, F.A.O., Expert on Hides and Skins, the following two 16 mm. films were shown.

1. Dutch silent film : Modern method of flaying of big animals.

2. Food and Agricultural Organisation film on " The Hide Or Skin " taken in North Africa.

After presentation of all the above papers, there was discussion on the subject and Mr. Soni in his concluding remarks stated that he had conducted some experiments by the oral administration of Gammaxane to goats for control of ticks and warbles.

TECHNICAL SESSION IV.

30th March 1957. (Forenoon).

The fourth and last session of the symposium was presided over by Dr. Bertie A. D'Souza, Principal, Madras Veterinary College, Madras. The following papers were presented :

1. Notes on raw hides and skins from picker manufacturers' point of view—By S. G. Desai, Pickers Ltd., Ahmedabad.

2. Flaying, its present position and some observations—By F. D. Wilson, Dept. of flaying and meat inspection, Madras Veterinary College, Madras.

3. Economic utilisation of carcasses in India—By F. H. Hoek, F.A.O., Expert to the Government of India.

4. Economic utilisation of carcasses in India—By N. Haq, Hide Development Officer, U.P.

5. Bye-products from the slaughter houses and their utilisation—By Dr. L. Hira, Superintendent of Markets and Slaughter Houses, Bombay Municipal Corporation, Bombay.

6. Production of Hides and Skins—By Palit, Dept. of Industries, Government of Orissa.

7. Salt curing of fresh slaughter house hides—By V. P. Pandit and Y. V. Lele, Western India Tanneries Ltd., Bombay.

8. Investigation on the preparation of neatsfoot oil—By M. Banerjee and P. K. Sircar, Bengal Tanning Institute, Calcutta.

9. Products of slaughter houses and their uses—By S. Venkataraman, Central Leather Research Institute, Madras.

10. Some problems relative to the raw hides and the suggested remedies—By S. Venkataraman, Central Leather Research Institute, Madras.

11. Recommendations of the Special Committee—By Mr. Rutherford, Gordon Woodroffe Leather Mfg. Co. (Private) Ltd., Pallavaram.

After the presentation of these papers, there was discussion on the subject. Dr. Bertie A. D'Souza in his concluding remarks said that the flayers in villages should be taught the proper methods of flaying and they should also be encouraged by giving proper tools. With co-ordination with the Community Development Centres, a proper scheme may be drawn up for training flayers which would ultimately lead to the production of good hides and skins in the Indian tanning industry. Animals in India, he said, were reared mainly for milk and draught purposes unlike in other

foreign countries for meat and beef. Hence he stressed that more care should be exercised for production of quality hides.

Dr. S. K. Barat, Senior Scientific Officer, Central Leather Research Institute, Madras, gave his concluding remarks and Mr. N. S. Mani, Publicity Officer of the Institute proposed a vote of thanks.

SPECIAL COMMITTEE FOR RAW HIDES AND SKINS

The delegates attending the symposium on Raw Hides and Skins—Curing and Preservation noted with great concern the rapidly deteriorating situation regarding the quality of hides and skins produced in India. Since hides and skins as such and in the form of tanned leather played an important part in the national economy and were responsible for earning a substantial portion of foreign exchange, it was felt that time had certainly come for re-examining the whole question of improvement of raw hides and skins in the light of newer knowledge gained by experience and research. In this context, it was felt that attention should be immediately focussed on the eradication of extensive damage caused by warbles, beetles, ticks, etc.

The General Body appointed a special committee under the Chairmanship of Mr. W. A. Rutherford of Messrs. Gordon Woodroffe Leather Manufacturing Co. (Private) Ltd. to make suitable recommendations for implementing the schemes of effecting all-round improvement in the production of hides and skins.

The other members of the Committee were :

Mr. N. M. Anwar, Hony. Secretary, Southern India Skin and Hide Merchants' Association, Madras.

Mr. G. S. Srinivasa Iyer, Messrs. Srinivas & Co., Madras and a Vice-President of the above Association.

Mr. Nagappa Chettiar of Messrs. India Leather Corporation, Madras and another Vice-President of the above association.

Mr. C. K. Duraivelan of Messrs. Kalyanam & Co., Madras.

Mr. A. J. Sharman of Messrs. Gordon Woodroffe Leather Manufacturing Co. (Private) Ltd., Madras.

Mr. B. R. Sen Gupta of Messrs. Bata Shoe Co. (Private) Ltd., Batanagar.

Mr. F. H. Hoek, F.A.O., Expert on Hides and Skins, Government of India.

Dr. S. K. Barat, Senior Scientific Officer, Central Leather Research Institute, Madras as non-member Secretary of the Committee.

The Committee which held two sittings on March 29 and 30, expressed the opinion that " an Indian Hides and Skins Improvement Society " should be formed so that necessary steps could be taken through legislation or otherwise in the interests of the industry including improvement of the species of livestock by cross-breeding, etc. ; promoting better health among animals by eradicating diseases, pests, etc. ; collecting old and useless animals and founding suitable animal homes at central places in the country ; improving the technique of flaying and curing of hides and skins from slaughtered and fallen animals ; properly utilising the carcass bye-products of fallen animals ; assisting villagers to obtain the best value for the hides, skins and offal from the carcasses which will encourage them to take better care of animals while they are alive ; improving the method of collection and storage of fallen and slaughtered hides and skins ; improving the existing transport facilities and co-operating with and co-ordinating the activities of allied organisations at governmental and non-governmental levels.

The Committee also felt that much of the work could be advantageously channelled through the rural extension services in the Five-Year Plan and underlined the desirability of gearing its activities with the existing machinery of these services as far as possible. The Committee was of the opinion that the part of the cess accruing from the export of hides and skins should be utilised for financing development projects in the field of hides and skins production.

The above recommendations were put before the house by the Chairman of the Committee, Mr. Rutherford. Mr. A. B. Narayana Mudaliar proposed and Mr. Jamal Mohamed, Hides and Skins Merchant, seconded and the same was adopted.

" AT HOME " TO DELEGATES

The Central Leather Research Institute was " At Home " to the delegates and hides and skins merchants who participated in the symposium on Raw Hides and Skins—Curing and Preserva-

tion on Friday, the 29th March 1957. Hon'ble Shri K. Kamaraj, Chief Minister of Madras, was the Chief Guest on this occasion.

Dr. Y. Nayudamma, Assistant Director-in-charge, Central Leather Research Institute, welcomed the Chief Minister.

Mr. N. M. Anwar, Hony. Secretary, Southern India Skin and Hide Merchants Association, thanked the Institute for the help given to the tanning trade. Mr. M. A. Muthia Chettiar (Rajah of Chettinad), member of the Executive Council of the Institute, said the Institute did very good work in giving the necessary advice and guidance to tanners. Mr. Nagappa Chettiar said the flayers and curers should follow the scientific method in curing and take the necessary advice from the Institute. Mr. C. K. Duraivelan thanked the Institute for having arranged the symposium.

Mr. Kamaraj, praising the work done by the Institute, said the symposium gave opportunities even to laymen to express their views which were sometimes valuable and could be utilised with advantage by the Institute. He pointed out the importance of the industry being built up on modern lines for then only would there be good scope for more sales. He assured all help to the trade and asked the Institute to advise the Government on the needs of the Industry to enable them to render the necessary aid.

Dr. Nayudamma proposed a vote of thanks.

The day's proceedings were rounded off with a dance recital by Kumari Rita Das, member of the staff of the Institute and her party.

ABSTRACTS OF THE PAPERS PRESENTED AT SYMPOSIUM ON HIDES AND SKINS—CURING AND PRESERVATION

1. Future Development of Indian Tanning Industry.

By J. J. Walters, Guildford, Surrey, England.

General remarks on the future development of the Indian Tanning Industry with special reference and suggestion for Hide Improvement Scheme.

2. Collection of raw goat skins on Co-operative basis.

By R. V. Sovani.

Flaying of hides and skins as done in remote villages is not usually done with proper care and attention due to the want of necessary equipments and this leads to a deterioration in the value of the stock. It has been suggested as to how this could be improved by proceeding on a Co-operative basis.

3. Note on collection of raw hides and skins and suggestions for improved methods.

By Dr. L. M. Hira, Superintendent of Markets, Greater Bombay.

Want of proper knowledge and necessary equipment for flaying are causes leading to the lowering of the value of hides and skins and ways to improve this have been suggested.

4. Supply of quality raw hides and skins to Tanners Co-operative Societies etc.

By C. V. Gangal, Tanning and Leather Expert, Industrial Co-operatives and Village Industries, (B. S.), Poona.

Development schemes to improve flaying of hides and skins have been advanced.

5. Improvement of Raw Hides and Skins.

By N. S. Mani, Central Leather Research Institute, Madras.

6. Brining of animal hides—its merits and demerits.

By S. C. Nandy, Central Leather Research Institute, Madras.

The brining process of curing is described with some of its controlling factors. Although this process is carried out practi-

cally in some parts of America, its superiority over that of salting process is well recognised in different parts of the world. The merits and demerits of the process in comparison with that of the salting process are discussed.

7. Preservation of Indian goatskins for export.

By M. Dempsey and B. N. Haines. The British Leather Manufacturers' Research Association, Milton Park, Egham, Surrey.

120 doz. drysalted skins and 120 doz. Calcutta kills were used in this experiment. Dry cured skins were given 0.01% or 0.1% sodium pentachlorophenate on their dry weight, and corresponding numbers were kept as controls without antiseptic. Some of the skins were sent to England immediately and some were stored in India through the monsoon. Judging by the number of good glace kid leathers made from the control skins (i.e., as normal production, with no antiseptic) it was concluded that drysalting, so long as the skins are not to be stored, can give satisfactory cure but improvement in method of cure of Calcutta kills is desirable since they did not give a full percentage of satisfactory leather even when sent immediately to England, while after storage in India during the monsoon the loss was considerably aggravated.

Under English conditions of storage the drysalted skins kept satisfactorily, but the Calcutta kills deteriorated. Under Indian climatic conditions there was appreciable deterioration of all types of skins that had not been treated with antiseptic. The pentachlorophenate improved the resistance of the drysalted skins but not the Calcutta kills owing, it is considered, to the poor condition of the cured skins before they received the antiseptic : treatment of the cured skins with 0.1% pentachlorophenate will prevent deterioration if the original bacterial condition of the skins was good.

8. Raw Hides and Skins—Curing and Preservation.

By D. Woodroffe, Principal, Northampton College of Leather Technology, England.

Some of the prevailing conditions which affect the quality of raw hides and skins have been discussed. Addition of bactericides for effective curing and preservation has been suggested.

9. Factors affecting the salting of raw hides and skins and their preservation.

By S. C. Nandy & S. N. Sen, Central Leather Research Institute, Madras.

Some of the problems involved in salting hides and skins and their subsequent preservation are dealt with and the conditions suitable for an ideal cure and preservation are suggested. Factors like nature, quality and quantity of salt to be used and the effects of temperature, moisture and humidity on preservation and the latest developments in increasing the curing efficiency of common salt by suitable additions are discussed.

10. Some aspects of curing practice.

Ministry of Commerce & Consumer Industries, Small Industries Service Institute.

Factors which are expected to affect the efficiency of curing processes have been discussed. The advantage of brining process over salting has also been suggested in case of hides of poor substance.

11. Curing of hides and skins.

By M. K. Bhagwat, State Industrial Co-operative Association Ltd., 9-Bake House Lane, Fort, Bombay-1.

Suggestion on methods of salt curing have been made.

12. Some consideration on the utilization of bittern salt in curing.

By S. C. Nandy & S. N. Sen, Central Leather Research Institute, Madras.

The possibility of utilizing the bittern salt which are considered as commercial waste in hide curing is discussed. Sodium carbonate and not the sodium sulphate present in bittern salt is supposed to be responsible for hard-cure i.e., such cured skins cannot be soaked back to proper condition. Some suggestions are made to render the bittern salt suitable for curing hides and skins.

13. Pickling hides and skins as a method of preservation.

By M. K. Bhagwat, State Industrial Co-Operative Association Ltd., 9-Bake House Lane, Fort, Bombay-1.

Suggestions on methods of acid pickling have been made.

14. PRESERVATIVE EFFICIENCY OF COMMON SALT IN ADMIXTURE WITH SODIUM PENTACHLOROPHENATE.

By S. C. Nandy & S. N. Sen, Central Leather Research Institute, Madras.

The efficiency of sodium pentachlorophenate as a suitable addition to common salt to increase its bacteriostatic action was studied. The addition of 0.5 per cent of Sod. pentachlorophenate to curing salt or 0.2 per cent on the raw weight of the skin was found to be advantageous in curing and preserving skins. Sod. pentachlorophenate cannot entirely prevent the growth of 'red-heat' organisms even if added in the proportion of 2.0%.

15. SOME ASPECTS OF THE BIOCHEMISTRY OF RAW HIDES AND SKINS.

By S. M. Bose, Central Leather Research Institute, Madras.

A review of the researches carried out on the biochemical aspects of raw hides and skins will be presented with a special reference to the work done in the Biochemistry Laboratory of the Central Leather Research Institute, Madras, on skin proteins and enzymatic reactions on skins and hides.

16. STUDIES ON THE PREVENTION OF INSECT DAMAGE TO HIDES AND SKINS.

By R. Bhaskaran & S. N. Sen, Central Leather Research Institute, Madras.

The usefulness of benzene hexachloride for combating insect damage in hides and skins has been discussed and an effective concentration of B. H. C. for this has been determined.

17. HISTOLOGICAL STUDY OF THE EFFECT OF POSTMORTEM CHANGES ON THE LEATHER FORMING QUALITIES OF CALF SKIN.

By S. K. Sarkar and S. K. Mitra, Central Leather Research Institute, Madras.

Postmortem changes taking place in uncured calf skin under Indian climatic condition has been studied and the effect of putrefactive processes on the physical properties of chrome tanned leather has been shown.

18. A NOTE ON THE DAMAGE CAUSED TO THE SKIN OF CATTLE, SHEEP, BUFFALOES AND GOATS BY PARASITES.

By U. S. Alwar, Lecturer in Parasitology, Madras Veterinary College, Madras.

The damage caused by Arthropod, Helminth and Protozoan Parasites to the skin of living cattle, buffalo, sheep and goat are described and preventive methods suggested.

19. DEFECTS IN RAW HIDES AND SKINS AND SUGGESTIONS FOR IMPROVEMENT.

By The Director of Animal Husbandry, Andhra Pradesh.

Hide and Skin defects caused by improper care and management of livestock prior to slaughter and during flaying are discussed with suggestions for improvement.

20. PARASITIC SKIN DISEASES OF ANIMALS AND THEIR EFFECTS.

By R. Bhaskaran, Central Leather Research Institute, Madras.

Different parasitic skin diseases affecting Indian cattle, sheep and goats and the defects they produce on the finished leather are described.

21. VIRAL, BACTERIAL AND OTHER DISEASES CAUSING DAMAGE TO LIVE HIDES AND SKINS.

By K. P. Chandrasekharan and B. Narasinga Rao, Madras Veterinary College, Madras.

The damage caused to the hides and skins of the living animals by some viral, bacterial and other conditions are described.

22. CERTAIN PARASITIC INFECTIONS OF CATTLE INVOLVING THE SKIN.

By M. Anantharaman, Research Officer (Helminthology), Madras Veterinary College, Madras.

Certain injuries caused by some nematodes (round worms) to the skin of the living cattle that are likely to reduce the value of the hide are described.

23. SOME DEFECTS IN RAW HIDES AND SKINS AND SUGGESTIONS FOR THEIR IMPROVEMENT.

Dr. B. N. Soni, Research Officer, Indian Veterinary Research Institute, Izatnagar. (U.P.).

24. THE IMPACT OF ANIMAL DISEASES ON LEATHER MANUFACTURE..

By R. Bhaskaran, Central Leather Research Institute, Madras.

The significance of the bacterial and viral diseases of animals to the leather manufacturer is discussed both from the point of

view of health hazard to tannery workers and the quality of leather produced.

25. Investigation on the preparation of Neatsfoot oil.

By M. Banerjee, Superintendent and P. K. Sarkar, Liaison Officer, Bengal Tanning Institute, Calcutta.

Fresh cattle legs were cleaned, scraped, washed, chopped and boiled with water in an open pan for about 12 hours. The supernatent oil was skimmed off and treated with brine and left overnight. Next morning the oil was separated and again treated with cold water and left for two days. Then the oil was separated and filtered which showed a yellow colour and had a bland taste. On analysis, the oil was found to compare favourably with imported stuff.

26. Bye-products from the slaughter houses and their utilisation.

By Dr. L. M. Hira, Superintendent of Markets and Slaughter Houses, Bombay Municipal Corporation.

The important of utilizing useful bye-products in slaughter houses starting from animal glands to hooves, horns, hair etc. has been discussed.

27. Products of slaughter house and their uses.

By S. Venkataraman, Central Leather Research Institute, Madras.

The products that can be obtained from a slaughter house is discussed and the various uses to which they can be put to are given.

28. Salt curing of fresh slaughter house hides.

By V. P. Pandit and Y. V. Lele, Western India Tanneries Ltd., Bombay.

The considerable advantage that has been derived by modifying the process of salting green hides from slaughter houses has been discussed in the paper.

29. Production of hides and skins.

By the Department of Industries, Govt. of Orissa.

Methods for improvement in flaying operations as well as proper storage and transport facilities for hides and skins have been discussed.

30. Flaying, its present position and some suggestions.

By F. D. Wilson, Department of Flaying and Meat Inspection, Madras Veterinary College, Madras.

The flaying methods as followed at present are described and suggestions for the improvement of hides and skins by proper flaying methods are discussed.

31. Some problems relative to the raw hides and the suggested remedies.

By S. Venkataraman, Central Leather Research Institute, Madras.

The various defects in the methods of flaying curing, collection, distribution, transport and marketing of raw hides are described with suggestions for improvement.

32. Notes on Raw Hides and Skins from Picker Manufacturers point of view.

By S. G. Desai, Pickers Ltd., Ahmedabad-6.

Suggestions for the improvement and conservation of the hide stock from the moment it is removed from the dead animal are discussed in the paper.

33. Export of goat skins.

By Calcutta Hide and Skins Shippers Association.

Suggestions for the development of the export trade of goatskins and the ways to explore the various resources of the raw material are discussed.

34. Manual for proper flaying, curing and carcass utilisation.

By F. H. Hock, F.A.O. Expert to Govt. of India, Hides, Skins and Leather, New Delhi.

35. Discussion on Curing and Preservation.

By B. R. Sengupta, Bata Shoe Co., Patna.

36. Economic utilisation of Carcasses in India.

By N. Haq, Hide Development Officer, U.P.

37. Improving the cattle wealth especially Goat and Sheep

By A. B. Narayanan, Madras.

CLASSIFIED ABSTRACTS

RAW MATERIALS

NEW METHOD FOR PREVENTING BACTERIAL DAMAGE ON HIDES. *By G. H. Green, Lea. Tr. Rev.* **121**, 3670, 251, (1956). Though cheap, effective and commonly used, sod. hypo chlorite has the disadvantages such as combining with skin protein thus incapable of preventing the growth of bacteria already present in the skin. It also combines with wool and is thus not useful for soaking sheep skins. Other antiseptics such as cryselic acid, chlorinated phenol etc. have the same disadvantages. The new method which is patented consists of sod. chlorite which possesses none of the above disadvantages. Stable in acidic and basic solutions ; diffuses into skin and reacts with protein slowly ; does not react with wool hence makes itself useful in soaking wool sheep skins ; easily soluble in water and hence can be easily washed ; waste pieces can be used for gelatin manufacture ; no danger of formation of stains or insoluble soaps in later processes ; can be used with enzymes in enzyme unhairing ; it is odourless and will not cause dermities. Analytical control, both qualitative and quantitative, is simple. Suitable method of analysis for works control is given. Some precautions to be observed in the use of sod. chlorite are described.

K. S. R.

PROBLEMS OF CURING IN THE SKIN AND LEATHER INDUSTRY. *W. Hausam, Rev. tech. Industr. Cuir,* **47**, 47-51, (1955). *Via. J. Appl. Chem.* **6**, 11, 408, (1956). The addition of *p*-chloro-*m*-cresol to curing salt is recommended as a bactericide for skins ; it can also be used to preserve hide glue. A table indicating the efficiency of various fungicides is discussed.

TANNING

VEGETABLE TANNING

THE ISOLATION OF SYRINGIC ACID FROM MIMOSA TANNING EXTRACT. *H. G. C. King. Chem & Ind. No.* 26, 658, (1956). Gallic acid and one other acid showed by the acidic fraction of ether-ellyl acetate fraction of comml. Mimosa Tanning Extract (*Acacia Mollissima*) were separated by chromatography using acetic acid as solvent. The m.p. and u.v. spectrum of the recrystalised other acid, produced as above, were found to be similar to syringic acid. The properties of the acid, giving red-brown colour with $FeCl_3$ solution, R_F value of 0.55/0.84, and wine red colour changing to yellow on spraying chromatogram with bis-diazotized benzidine solution were similar to syringic acid. Identical test with an ether extract of the bark showed that it was not produced during the manufacture of the extract.

K. S. R.

THE RELATIVE CONFIGURATIONS OF CATECHIN AND EPICATECHINE. *A. J. Birch, A. V. Robertso and J. S. Clark-Lewis. Chem & Ind. No.* 26, 664, (1956). The epimerization of 3-hydroxyl group in the conversion of (+) catechine to (+) epicatechin by heating with mild alkaline solution was evidenced by this study and experiments were designed to establish the configurations of the catechines.

K. S. R.

THE ISOLATION FROM *ACACIA MOLLISSIMA* OF A CRYSTALLINE LEUCO ANTHOCYANIDIN. *H. H. Keppler. Chem. & Ind.* 18, 380, (1956). Acetone extract of black wattle heartwood is suitably fractionated and purified on a column of 'Solka Floc' when a light brown hygroscopic material which on further purification gives colourless needles, m.p. 127° (decomp). Constants for this are given. Acetyl and methyl derivatives of this substance are prepared. It gives a dark red precipitate with 10% aqueous HCl, while with 10% ethenolic HCl, a red solution is obtained. The leuco anthocyanidin is envisaged as 5:3′:4′-trihydroxy-flavan-3:4-diol, or 7:3′:4′-trihydroxy-flavan–3:4 –diol or 7:8:4′–trihydroxy–flavan -3:4 diol. Further work is reported to be in progress.

K. S. R.

CHROME TANNING

ADVANTAGES OF TANNING WITH MASKED CHROMIUM SALTS. *E. Belubsky and I. H. Hrebicek, The Lea. Tr. Rev,* **121**, 3670 (1956). The advantages of using masked chromium salts for tanning and that of tanning which involved masking effect with combined tannages such as chrome retan, semi chrome, silicon chrome are briefly described. Recipes for the preparation of liquors using sod. formate and sod. acetate, for chrome retanning using mimosa extract and for silicon chrome tannage are given.

K. S. R.

PROCESSES AFTER TANNING

DYEING PASTEL SHADES ON VEGETABLE TANNED LEATHER. *D. H. Tuck. The Lea. Tr. Rev.* **122**, 3183, 278, (1956). Leathers after stripping with mild alkalis or solvents to remove dark coloured oxidised surface tannins, are to be bleached and retanned with combination type syntan. Use of Sodium sulphite for bleaching or sulphited extract for the tanning is to be avoided as SO_2 present can cause uneven bleaching of the dyed leather. Small amounts of acid dyedstuff with long float of water and temperature below 30°C is usually used for pastel dyeing. Addition of 3% neutral syntan before or during dyeing helps levelling. Addition before dyeing produces maximum levelling effect, increases penetration and decreases surface intensity. Anionic assistants such as sulphated oils, anionic surface active agents are used but they change water repellency and the feel of the leather. Nonionic assistans are useful in greasy seep skins. ¼-½% of buffer salt such as am. acetate, acetate and citrate of sodium can be added. Using of pale and med. coloured dyestuff at several intervals will aid the production of pastel shade.

K. S. R.

THEORIES

STUDIES ON THE COORDINATE BOND. IV. The MECHANISM OF FORMATION AND OF DISSOCIATION OF THE TRIS-(2, 2′-DIPYRIDYL) IRON (II) COMPLEX. *P. Krumholz. J. Phy. Chem.* **60**, 1, 87, (1956). The rate of formation of the tris-(2,2′-dipyridyl)-iron (II) complex was determined in solutions up to 2.2M in hydrochloric acid and compared with the rate of dissociation of that complex determined under similar conditions. The rates of those two reactions both show the same dependence on the hydrogen ion concentration. The experimental rate laws are mathematically equivalent to expression derived by means of the steady state approximation applied to a reaction scheme involving intermediates in which 2, 2′-dipyridyl behaves as a monodentate group. It was found that 2, 2′-dipyridyl behaves in strongly acid solutions as a biacid base and the value of the corresponding dissociation constant is reported.

MECHANISM OF GELATION OF GELATIN. INFLUENCE OF CERTAIN ELECTROLYTES ON THE MELTING POINTS OF GELS OF GELATIN AND CHEMICALLY MODIFIED GELATINS. *Jake Bellow, Helene C. A. Riese and Jerome R. Vinograd. J. Phy. Chem.* **60**, 9, 1299, (1956). The effects of series of salts and acids on the melting points of gelatin gels have been studied. The effects of ions, of the same or opposite charge, are additive. There is a correlation between the binding of ions, as indicated by pH changes, and their effects on the melting point. However, by the use of amino-acetylated gelatin and a guanidino-nitrated hydroxyl sulfated gelatin, it is shown that binding of anions at amino, guanidino or hydroxyl groups is not responsible for melting point changes. By the use of carboxyl-esterified gelatin and hydroxyl-acetylated gelatin, it is shown that binding of cations at carboxyl or hydroxyl groups is not the cause of melting point reduction. Iron (III) ion, at low concentration, raises the melting point by inter- or intramolecular cross-linking through the carboxyl groups. In agreement with previous reports polarizable anions are effective melting point reduces with diiodosalicylate being the most potent observed.

SWELLING OF PROTEIN MOLECULES IN SOLUTION AND THE α-β TRANSFORMATION. *Terrell L. Hill. J. Phy. Chem.* **60**, 3, 358, (1956). The suggestion of Yang and Forster that bovine serum albumin swells at low pH because of an α-β transformation is examined theoretically using a necessarily approximate treatment. The conclusion reached is that the Yang-Forster mechanism is a reasonable possibility. The theory, in its present form, predicts a salt effect of opposite sign to that found experimentally. Refinements in the theory will presumably remove this discrepancy.

TESTING

A SIMPLE DIP TYPE SATURATED CALOMEL ELECTRODE FOR POLAROGRAPHY *P. O. Kane, Chem. & Ind. No.* 16, 296, (1956). A new type of electrode to be useful for polarography with schematic diagram is described. The advantages are that it does not appreciably contaminate the solution under investigation ; its potential is reproducible

and the polarization by anodic current upto 20 micro amp has been found to be 2 mv and at 50 micro amp only 9 mV. The disadvantages are that Ohmic potential drop is to be corrected to half wave potential and 5 ml of solution is required for testing.

K. S. R.

DETERMINATION OF UREA-FORMALDEHYDE RESINS IN COATINGS VIA UREA ANALYSIS. *M. H. Swann and G. G. Esposito. Anal. Chem.* **28**, 12, 1984, (1956). The release of ammonia from urea-formaldehyde coating resins on treatment with alkali is characteristic. It provides a rapid quantitative means of analyzing coatings for urea resin modification or of determining urea in urea-formaldehyde resins.

SILICON AND ALUMINIUM CONTENT OF CHROME LEATHER. *C. A. Danon Rev. tech. Industr. Cuir,* **47**, 168-170, (1955) *Via J. Appl. Chem.* **6**, 11, 408, (1956). Causes of inaccuracies in determination of Al in chrome leather are chiefly the presence of Si, and the solubility of Al $(OH)_3$. It is recommended that Si be determined in the ashed leather by treating with HCl or HF/H_2SO_4.

PATENTS

SOLVENT CHROME TANNAGE UNDER PRESSURE. (*French Pat.* 1, 093, 089). *Through Lea. Tr. Rev.* **122**, 3684, 312, (1956). According to the specification, skins are laid flat on a porous material (e.g. wool felt 6.3 mm. thick) which is supported on a metal seive ; above the skin is an impermiable sheet of rubber or polythene, with a hide in the centre through which liquids are introduced. A different press. is exerted between the porous surface being lower than that of the membrane. Acetone is passed through the skin under pressure until it is dehydrated ; the pelt is then tanned with a solution of 100 parts saturated chromic chloride in methanol and 500 parts acetone plus saturated aqueous ammonia. Tannage is claimed to be complete in a few minutes when the tanning solution remains green after passing through the skin.

BOOK NOTICES

"A HAND BOOK ON CHROME TANNING" *by D. Woodroffe, M.Sc., A.R.I.C., A.B.S.I.* 187 *pages, Published by Quality Books, Teignmouth, Devon, Price* 24*s. Via Lea. Tr. Rev.* **122**, 3678, 65, (1956).

This is a timely book that will be welcomed by the practical chrome tanner no less than by the student of leather science. The author is head of the department of leather manufacture, Northampton College of Technology, and knows his subject thoroughly.

As is set out in the preface, over 90 per cent of the upper leather used in shoe manufacture is reputed to be chrome tanned. This alone makes it essential that a book should be available that gives details of the practical methods used in chrome tanning and this is what the book sets out to do in a complete and comprehensive manner. The author's historical introduction provides a useful background to the modern methods used today in chrome tanning and some of this history is worth recalling. It was as long ago as 1858, nearly 100 years ago, that the

first experiments in chrome tanning were carried out by Knapp, who described the tanning properties of iron aluminium and chromium salts. His conclusions were that chromium salts produced a greyish blue leather which was too hard and tinny for commercial use. That put a halt to further experiments in chrome tanning for the next 26 years. Then, in 1884, Schultz, a New York wool chemist, was asked to produce a leather which would not rust corset steels. He applied chrome mordanting to the pelts and so discovered the two-bath chrome tanning process. With slight modification, this process continued in general use until about 1940 for the tanning of glace kid (or glazed goat). The discovery made by Schultz led to attention once again being directed to the possibilities of chrome tanning, attempts were made to produce chrome leather by means of basic chromium salts and the one bath chrome tanning process, originally discovered by Knapp, was developed and improved.

LATEST ADDITIONS TO LIBRARY:

1. Dodge—Chemical Engineering Thermodynamics.
2. Perry—Chemical Engineers Handbook.
3. Thimann—Life of Bacteria.
4. Sutton—Systematic Handbook of Volumetric Analysis.
5. Chatfield—Paint & Varnish Manufacture.
6. Block, Durran, Zweig—Paper Chromatography.
7. Bonner—Plant Bip-chemistry.
8. Sptringall—Structural Chemistry of Proteins.
9. Mallicllo—Protective and Decorative coatings Vols. 1-5.
10. Bracker—Chemistry of micro-organisms.
11. Daniels Alberty—Physical Chemistry.
12. Reillay, Rae—Physico-chemical methods : 2 vols.
13. Groggins—Unit Processes in Organic Synthesis.
14. Vilbrandt—Chemical Engineering Plant Design.
15. Klopsteg, Wilson—Human Limbs and their substitutes.
16. Tyler—Chemical Engineering Economics.

NOTES & NEWS

SALTING COMMISSION RECOMMENDS USE OF SALT WITH SODA AND NAPHTHALENE. LTR, **121**, 3676, 503 (1956).

I. H. A. T. I. S. salting commission was unanimous that that superior and more practicable method than others was by using salt with soda and Naphthalene. The Commission felt the widest publicity should be given to the value of such a mixture not only because it was known to overcome the growth of red heat and salt stain, but also because it was the most practicable method and was known in most countries. The method of applying the mixture is given in brief.

SINGAPORE HIDE BAN : Singapore Government has announced a ban on the import of hides, skins and offal from North and South America " to prevent the introduction of animal diseases into the colony ".

—*L. T. R.* **122**, 3681, 208 (1956).

WANTED ; A WARBLE KILLER : Surprise offer of 5 million francs by the French Government to the person who could find a product that would eliminate the warble fly provided a dramatic interlude during the annual congress of the International Hide and Allied Trades' Improvement Society in Paris, in September. Full details, including the rules of the award, are to be announced later.

—*L.T.R.* **121**, 3676, 500, (1956).

C. L. R. I. NEWS

The Valedictory Address of the Association of Leather Technologists (an Association founded by the Students of the B.Sc. (Tech.) Leather Course of the Madras University) was delivered on 15th March 1957 by Mr. Nazir Hussain at 4-30 p.m., at the Central Leather Research Institute, under the presidentship of Dr. Y. Nayudamma, Assistant Director-in-charge, Central Leather Research Institute. Those present included leading tanners of Madras and Shri P. M. Sundaram, Secretary, Council of Scientific & Industrial Research.

The Scientific Advisory Board of the Central Leather Research Institute met on March 28th at the Institute (at 9-00 a.m.).

The C.L.R.I. Staff Club celebrated its Annual Day on the 30th March 1957. Mr. S. K. Mukherji, Chief Commercial Superintendent, Southern Railways, presided. Smt. Rekha Menon distributed the prizes to the winners in the various tournaments. More than 85 prizes were distributed. The function lasted till 9-00 p.m., with a delightful variety entertainment by the members of the Staff Club.

The Practical Demonstration No. 3 on the 'Manufacture of picking band leather' has been started on 5th April 1957 and is in progress.

The following firms participated in the exhibition organised during the symposium on "Raw Hides and Skins — Curing and Preservation" held in this Institute on 28th, 29th and 30th March 1957.

(i) Messrs. Gordon Woodroffe Leather Manufacturing Co. Ltd., Pallavaram.

(ii) The Chrome Leather Co. Ltd., Chrompet.

(iii) The Art Home, Bombay.

(iv) Leather Chemicals & Industries Ltd., Calcutta.

(v) Office of the Joint Registrar of Co-operatives and Village Industries, Poona.

Dr. Y. Nayudamma, Assistant Director-in-charge, visited the tanneries and the Indian Institute of Science, Bangalore on 10th and 11th March 1957.

Dr. Y. Nayudamma, Assistant Director-in-charge, attended a meeting between representatives of the Ministry of Heavy Industries and of the Council held in New Delhi on 27th March 1957.

Sri J. Budaga Rao, Senior Scientific Assistant attended the Symposium on 'High Polymers' held at Poona during Feb. 28th to March 1957.

We congratulate Shri A. Ganesan, Junior Scientific Officer, on his promotion as Senior Scientific Officer.

VISITORS

Name		*Date of Visit*
Shri Jashbai J. Patel	..	9—3—1957.
Members of the Finnish Goodwill delegation	..	11—3—1957
Mrs. A. J. John & H. E. Mr. A. J. John, Governor of Madras	..	14—3—1957
Mr. U on Pe, Burmese delegation	..	19—3—1957
Mr. Riccordo Longone	..	21—3—1957