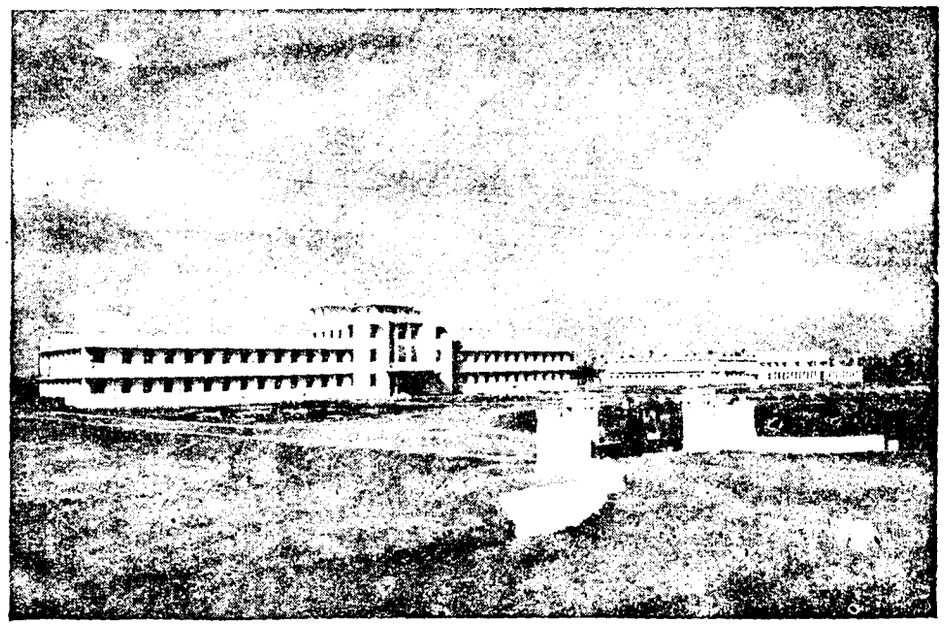
Bulletin of the

C. L. R. I. Madras

Feb. ~~1947~~ 1961

Bulletin of
The Central Leather Research Institute
Madras

Vol. VII FEBRUARY, 1961 347 No. 7



COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

INDIA

Bull. cent. Leath. Res. Inst., Vol. 7, No. 7, Pp. 301-349. Madras, February, 1961

Bulletin of
The Central Leather Research Institute

Vol. VII **FEBRUARY, 1961** **No. 7**

CONTENTS

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

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

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

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

PURIFICATION, CRYSTALLISATION AND PHYSICO-CHEMICAL PROPERTIES OF PROTEASE OF ASPERGILLUS PARASITICUS*

BY

S. C. DHAR & S. M. BOSE

Central Leather Research Institute, Madras

Received on August 4, 1960

SUMMARY

The purification and crystallisation of the protease of *A. parasiticus* were systematically studied. The nine different treatments which were found beneficial and which were given successively to the original enzyme extract resulted in a 99,510-fold purification of the protease. Crystallisation of the purified protease was also achieved. The physico-chemical properties of the crystalline protease were studied with reference to the action of the protease on skin and hide proteins, pH-activity relationship of the protease against different substrates, time-activity relationship, effects of varying enzyme concentration and temperature, ultraviolet irradiation and dialysis on proteolytic activity, pH-stability, heat inactivation and ultraviolet absorption spectrum of the protease.

Introduction

A good deal of work has been done on the purification, crystallisation and physico-chemical properties of certain microbial proteases. Purification of proteases of several bacteria, viz. *Clostridium histolyticum*,[1, 2] *Clostridium welchii*,[3] *Proteus vulgaris*,[4] *Bacillus mesentericus*[5] has been reported. Crystallisation of proteases from a few bacteria, viz., *Streptococci*,[6] *Bacillus amylolyquefacience*[7] and *Bacillus subtilis*[8, 9, 10] has also been effected. Proteases from *Aspergillus oryzae* and *Actinomyces* have been crystallised by Crewther and Lennox[11] and Miyake *et al*[12] respectively. Four proteolytic enzymes, viz., proteinase, carboxypolypeptidase, aminopolypeptidase and dipeptidase have been separated from the culture filtrate of *Aspergillus parasiticus* by Johnson.[13] Some of the physico-chemical properties of proteases of different organisms, viz., *Clostridium histolyticum*,[1] *Clostridium welchii*,[3] *Bacillus subtilis*,[9, 14]

* Based on Shri Dhar's thesis which was awarded the M.Sc. degree of Madras University.

Proteus vulgaris,[4] *Bacillus mesentericus*,[5] *Tetrahymena pyroformis* 'W'[15] and a number of species of *Alternaria*,[16] *Streptomyces*,[16] *Mortierella*[16, 17, 18] and *Gliocladium*[16] have been studied.

No work, however, has been reported so far on the purification, crystallisation or the physico-chemical properties of the proteases of *A. parasiticus*. In previous communications, it has been shown that the protease of *A. parasiticus* is capable of unhairing[19] and bating[20] skins and hides and is suitable for the production of enzyme depilants and bates for leather manufacture. It was, therefore, deemed of interest to make a thorough study on the physico-chemical properties of this enzyme The use of a highly purified or crystalline sample of the enzyme being essential for such a study, a systematic investigation was undertaken on the purification, crystallisation and the properties of the protease of *A. parasiticus*.

Experimental

Estimation of enzyme protein:

In order to standardise a routine method for the quantitative estimation of enzyme protein, the method of Lowry *et al*[21] was followed using twice crystallised trypsin (Nutritional Biochemical Corporation, Ohio) as the reference protein. The optimum wavelength for the colour reaction of crystalline trypsin by this method was found to be 750 mμ in a Beckman spectrophotometer (DU). 2 ml. portions of twice crystallised trypsin of different strengths were allowed to react with 10 ml. portions of alkaline copper sulphate solution and 1 ml. portions of diluted phenol-reagent solution. After 30 minutes, the developed colour was estimated in Beckman spectrophotometer (DU) at 750 mμ. The standard curve was drawn from the net optical density readings after deduction of readings for the reagent blank and the solution blank. For the estimation of the enzyme protein at different stages of purification of the enzyme extract, 2 ml. diluted enzyme solution was allowed to react with alkaline copper sulphate solution and the diluted phenol reagent solution in the same manner as before and the optical density was measured in the spectrophotometer at 750 mμ. From the net optical reading the enzyme protein was estimated with reference to the standard curve.

Estimation of specific activity:

The specific activity of the enzyme solution was calculated as the proteolytic activity per milligram of enzyme protein. For the estimation of proteolytic activity of enzyme solution at different

stages of purification, a measured volume of enzyme solution of suitable strength was added to 10 ml. of 2·5% egg-albumin (E. Merck) solution of pH 7·5 and the mixture was digested at 45°C for 30 minutes at the end of which the liberated tyrosine was estimated by the modified phenol-reagent method.[22]

Preparation of the original extract of crude enzyme from A. parasiticus:

The culture medium which was suggested in a previous communication[23] for the maximum production of the protease by *A. parasiticus* has been used in the present study. 25 ml. portions of culture medium (pH 6·0) composed of 4 gm. sucrose, 4 gm. peptone, 0·1 gm. dipotassium hydrogen phosphate, 0·1 gm. calcium chloride, 0·05 gm. sodium sulphate 0·05 gm. magnesium chloride, 0·001 gm. manganese sulphate and 0·001 gm. zinc sulphate per 100 ml. distilled water, were sterilized in a number of 300 ml. conical flasks and *A. parasiticus* was cultured under sterile conditions. The flasks were kept at 33°C for six days. The developed mold along with the entire culture medium was then triturated in a mortar with acid washed sand using 25 ml. bidistilled water for each flask. The extract was centrifuged at the rate of 3000 r.p.m. and the centrifugate was used as the starting material for the present investigation.

Purification of protease of A. parasiticus:

Effect of freezing and thawing on mold protease:—200 ml. original enzyme extract was allowed to freeze in a 250 ml. round bottomed flask which was surrounded with freezing mixture. When the solution was completely frozen, the flask was kept inverted. Four 50 ml. fractions were collected separately. The specific activity of each fraction was estimated. The results are presented in table I.

TABLE I

Concentration of mold protease by freezing and thawing.

Sample	Proteolytic activity (Mgm. tyrosine liberated per 5 ml. digestion mixture)	Mgm. enzyme protein in 5 ml. digestion mixture.	Specific activity
Original enzyme extract	0·56	5·6	0·100
1st fraction	0·65	2·30	0·283
2nd fraction	0·65	2·41	0·270
3rd fraction	0·18	3·36	0·054
4th fraction	0·09	3·08	0·029

The results show that the third and fourth fractions had mild proteolytic activity whereas the enzyme activity was concentrated in the first two fractions. This step was, therefore, quite useful in the purification of the protease.

Acetone precipitation of protease at different pH:

For 40 ml. portions of original enzyme extract which were buffered with 20 ml. portions of veronal buffer at different pH, 180 ml. portions of pure redistilled acetone were used. The acetone and the buffered enzyme solutions were cooled to about −8°C and +2°C respectively by means of freezing mixture and the buffered enzyme solution was added to acetone slowly under stirring. The samples of the precipitated enzyme were removed by centrifugation, dried under vacuum, reconstituted to 40 ml. portions with bidistilled water and the proteolytic activity was determined. The results are presented in table II.

TABLE II

Effect of pH on the acetone precipitation of mold protease.

pH of precipitation	Proteolytic activity (Mgm. tyrosine liberated per 5 ml. digestion mixture)
6·0	.. 0·20
6·3	.. 0·24
6·5	.. 0·31
6·75	.. 0·36
7·0	.. 0·40
7·3	.. 0·49
7·5	.. 0·51
7·75	.. 0·49
7·95	.. 0·44

The results show that the precipitate obtained at pH 7·5 has the maximum proteolytic activity.

Removal of nucleic acid from the enzyme extract by treatment with protamine sulphate:

In the course of a trial for standardisation of a routine method for the quantitative estimation of enzyme protein by the spectrophotometric method of Warburg and Christian,[24] portions of the enzyme solution at different stages of purification were suitably

diluted and the optical densities were measured at wavelengths 260μ and 28 mμ using the spectrophotometer. The results showed that the ratios of the optical densities at 280 mμ and 260 mμ at different levels of purifications were of the order of 0·8 to 0·9. As the ratio of optical densities at 280 mμ and 260 mμ is 1·80 for pure proteins and about 0·45 for pure nucleic acid, it appeared that the nucleic acid content of the enzyme extract was fairly high.

In order to remove nucleic acid from the enzyme extract of the mold, 1 ml. portions of 1·5 per cent protamine sulphate solution were mixed with 1 ml. portions of veronal buffer of different pH and these mixtures were mixed with 2·5 ml. portions of the centrifuged sample of the original enzyme extract. The mixtures were kept at room temperature for half an hour, centrifuged and the centrifugates were analysed for specific activity. The results are presented in Table III.

TABLE III

Treatment of protamine sulphate

Buffer pH	Proteolytic activity (Mgm. tyrosine liberated per 5 ml. digestion mixture)	Mgm. enzyme protein in 5 ml. digestion mixture	Specific activity
Centrifuged sample of the original enzyme extract			
—	0·50	5·01	0·100
5·0	0·47	2·60	0·181
6·0	0·52	2·46	0·211
7·5	0·50	2·14	0·234
8·5	1·42	1·07	1·327

The results showed that the treatment of enzyme extract with protamine sulphate at pH 8·5 was helpful for the removal of nucleic acid, the specific activity of the enzyme being increased quite appreciably. The removal of nucleic acid was also confirmed by measuring spectrophotometrically the optical density of the protamine sulphate treated enzyme solution of pH 8·5 at 280 mμ

and 260 mμ. The ratio of the readings at these wavelengths was found to be more than 1·4.

A series of other experiments on isolation and purification of protease of *A. parasiticus* was systematically carried out by adopting standard methods. The following observations are of interest: the precipitation of the protease at pH 7·5 using three volumes of acetone per one volume of enzyme solution resulted in the highest specific activity. Similarly, precipitation of the protease in the neighbourhood of pH 7·5 at various concentrations of ammonium sulphate showed that the precipitate obtained by full saturation with ammonium sulphate had the highest specific activity. Fresh calcium phosphate gel prepared by the method of Keilin and Hartree[25] was found to be very efficient for the adsorption of only the impurities of the enzyme extract at pH 7·5; the specific activity of the enzyme extract after removal of impurities by this gel was found to increase to a great extent. Based on these observations, the following procedure involving nine steps was adopted for purifying the protease of *A. parasiticus*.

Step I:—An enzyme extract was prepared from the entire culture medium and the developed mold as mentioned before. The extract was centrifuged at 3000 r.p.m. and the centrifugate was collected.

Step II:—The centrifugate (1 litre) was allowed to freeze in a round bottomed flask. The flask was then kept inverted, the frozen material was allowed to thaw and the first 500 ml. liquid was collected.

Step III:—The enzyme solution, the pH of which was adjusted to 7·5 was precipitated with 3 volumes of pure redistilled acetone by cooling the solvent and enzyme solutions to $-8°C$ and $+2°C$ respectively and adding the enzyme solution drop by drop to the acetone under constant stirring. The precipitated enzyme was removed by centrifugation, dried under vacuum and reconstituted to a suitable volume with bidistilled water.

Step IV:—The enzyme solution (100 ml.) was centrifuged at 15,000 r.p.m. and a clear centrifugate was collected.

Step V:—This centrifugate (95 ml.) was filtered through an ultrafine filter disc of an ultrafilter apparatus under vacuum and the clear filtrate was collected.

Step VI:—The filtrate (92 ml.) was mixed with 37 ml. of 1·5 per cent protamine sulphate solution and the pH of the mixture was adjusted to 8·5. After half an hour, it was centrifuged and the centrifugate collected.

Step VII:—The centrifugate (124 ml.) was mixed with 52 ml. fresh calcium phosphate gel (26·6 mgm./ml.) which was diluted twice with bidistilled water. The mixture was adjusted to pH 7·5, shaken for 10 minutes in a mechanical shaker, centrifuged and the centrifugate collected.

Step VIII:—To the centrifugate (165 ml.), the pH of which was adjusted to 7·5, solid ammonium sulphate was added till full saturation. The precipitated enzyme was removed by centrifugation and was dialysed for 3 days at 4°C against bidistilled water. The dialysed enzyme solution was made upto 55 ml. with bidistilled water.

Step IX:—Finally, from the above enzyme solution, the protease was precipitated by acetone under the same conditions as mentioned under step III. The precipitated enzyme was dried in a vacuum desiccator and powdered. The dry weight of the purified enzyme powder obtained was of the order of 25 mgm.

At each stage of purification, the protease solution was analysed for specific activity. The results obtained are summarised in table IV.

TABLE IV

Purification of protease of *A. parasiticus*

Treatments	Proteolytic activity (Mgm. tyrosine liberated per 5 ml. digestion mixture)	Mgm. enzyme protein in 5 ml. digestion mixture	Specific activity
Enzyme solution after			
Step I	0·58	5·70	0·102
Step II	0·72	2·44	0·295
Step III	0·63	0·62	1·016
Step IV	0·61	0·35	1·743
Step V	0·68	0·14	4·857
Step VI	1·60	0·031	51·61
Step VII	1·93	0·001	1,930·0
Step VIII	2·01	0·0004	5,025·0
Step IX	2·03	0·0002	10,150·0

Crystallisation of protease of A. parasiticus:

Different sets of experiments were conducted to crystallise the highly purified protease prepared on combining nine different steps of purification. However, the following method alone was successful in producing crystals from the solution of the purified enzyme.

5 mgm. portions of the purified protease were taken in a number of beakers (50 ml.) and were dissolved in 5 ml. portions of veronal buffer (which was prepared without using sodium chloride) of different pH values. Dry powdered ammonium sulphate was then added slowly to each beaker to incipient turbidity. The enzyme solutions were kept in the frigidaire at about 4°C. For each pH, a control experiment was run in an identical manner without using enzyme powder. After about four days, crystals were found to appear from the enzyme solution of pH 8·4. Crystals obtained were examined under dark ground microscope, a photomicrograph of the crystals was taken (fig. I). The proteolytic activity of the crystals was estimated after dialysis of the

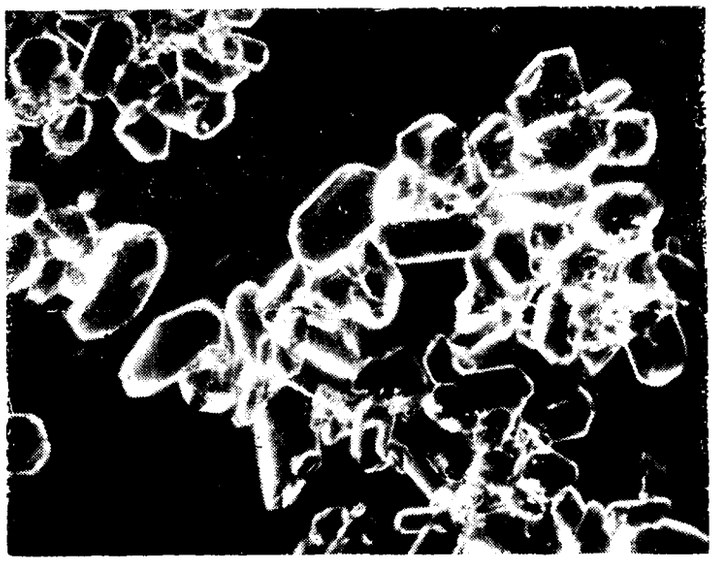


Fig. I: Crystals of protease of *A. parasiticus.* × 130·0

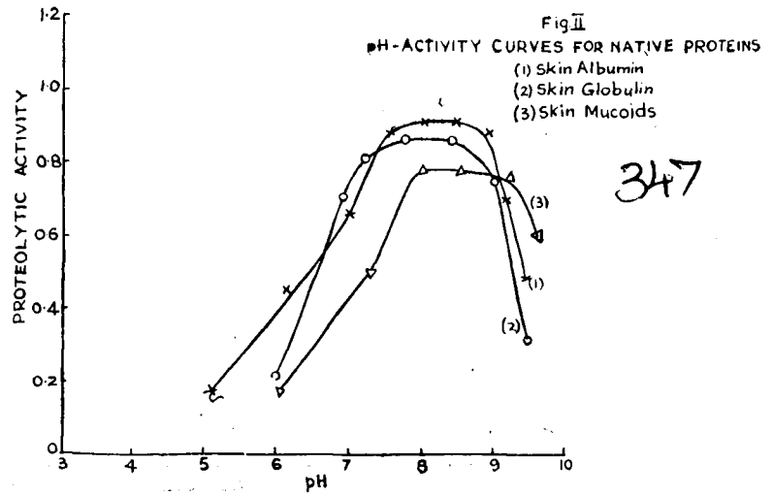
enzyme solution. It was observed that the proteolytic activity of the crystals was of the same order as that of the purified protease. After recrystallisation of the enzyme, it was, however, observed that there was no further increase in proteolytic activity. The twice crystallised protease was examined by the paper electrophoretic method of Durrum[26] which showed that the enzyme contained only one component.

Properties of the protease:

The crystalline protease prepared from *A. parasiticus* and stored in the frigidaire was used for studying its properties. Each time fresh enzyme solution in bidistilled water was prepared in suitable concentration and dialysed for 24 hours against bidistilled water at 4°C.

Effect of the protease on different proteins at different pH:

Although the practical unhairing and bating experiments did not show any adverse effect on collagen, it was thought desirable to study the effect of the crystalline protease of *A. parasiticus* on collagen and other skin proteins. The individual skin proteins of both fibrous and non-fibrous type were, therefore, prepared and the action of the enzyme on each protein was studied under different pH conditions. Collagen was prepared from the butt portion of a fresh buffalo hide by the modified method of Joseph and Bose.[27] Keratin was prepared from clipped wool and elastin from fresh *ligamentum nuchae* which was isolated from the neck of a slaughtered cow, by the methods adopted by Bose *et al.*[28] Reticulin was prepared from fresh lymph-nodes of cows by the method followed by Joseph and Bose.[27] Albumin, globulin and mucoids were prepared from a fresh goat skin by the method

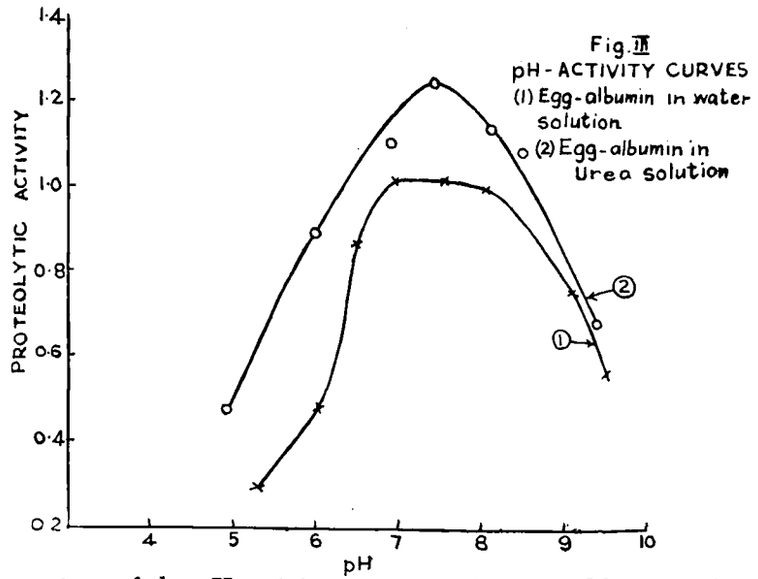


adopted by Bose *et al*[28] with the modification that albumin was precipitated from the water extract of the minced skin by acidification with acetic acid at pH 5·0 and the precipitate was recovered by centrifuging and dried in a vacuum desiccator.

With a view to studying the action of the enzyme on skin albumin, globulin and mucoids under different pH conditions, 20 ml. portions of 2·5 per cent solution or fine suspension of each protein in bidistilled water suitably buffered with veronal buffer at different pH values were allowed to be hydrolysed at 40°C for 25 minutes by 0·03 ml. of protease solution (2 mgm./100 ml.) and the degree of hydrolysis was determined by the phenol reagent method as modified by Bose and Madhavakrishna.[29] The results are presented in fig. II.

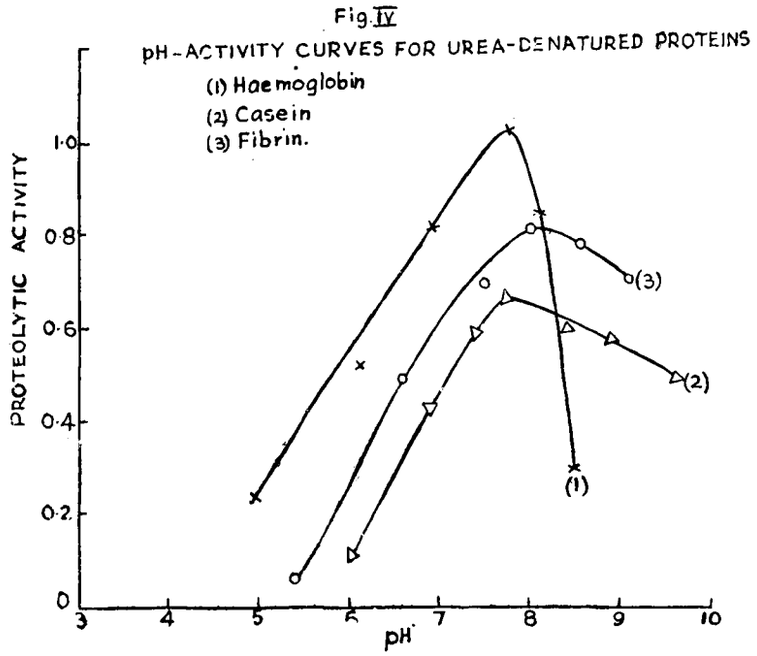
As collagen and reticulin do not contain appreciable amount of tyrosine, the effect of the enzyme on the fibrous proteins viz., collagen, reticulin, elastin and keratin under different pH conditions was studied by the non-protein nitrogen method of Northrop[30] as modified by Bose and Madhavakrishna.[22]

Anson,[31] and Krishnamurthi and Subrahmanian[32] observed that definite pH optima of certain proteolytic enzymes could be obtained only against urea-denatured proteins. In the case of present enzyme, it was, therefore, thought desirable to determine



the nature of the pH activity on a typical protein like egg albumin (E. Merck) with and without urea-denaturation. For enzyme estimation under different pH conditions, the same procedure as adopted in the case of globular proteins was followed except that in the case of urea-denatured protein, 3*M* urea solution was used instead of bidistilled water. The results are presented in fig. III.

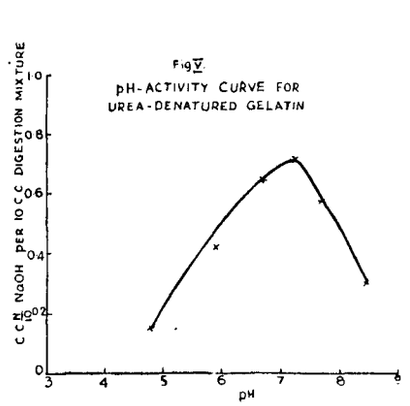
The results show that in the case of the present enzyme also a smooth pH-activity curve with a definite pH optimum could be obtained only against urea-denatured protein. Hence while studying the digestibility of a few other typical proteins like haemoglobin, casein, fibrin and gelatin by this enzyme under different pH conditions, the urea-denatured protein was used as the substrate. In the case of these proteins also the same procedure as adopted for urea-denatured egg albumin was followed. As haemoglobin or fibrin was not completely soluble in urea solution, these substrates were used in the form of fine suspension. Due to gelatin being not quantitatively precipitated by trichloroacetic acid, the proteolytic activity against this substrate was estimated by the formol titration method of Northrop[30] as modified by Bose and Madhavakrishna.[22] The results are presented in figs. IV and V.

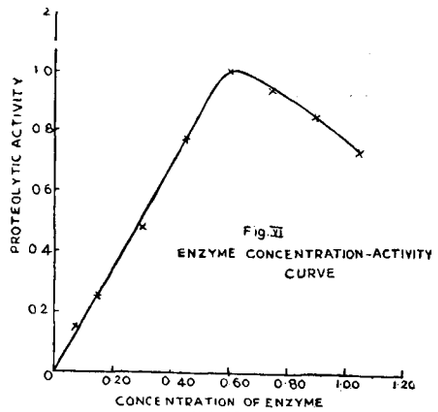


As egg albumin (E. Merck) was a standard protein soluble in water at different pH and as the hydrolysis by the enzyme was also found to be of higher order, further studies on the physico-chemical properties of the protease were carried out against this protein.

Effect of varying enzyme concentration on proteolysis:

In order to study the enzyme concentration-activity relationship, 20 ml. portions of 2·5 per cent egg albumin solution in bidistilled water buffered with veronal buffer at pH 7·45 were digested for 25 minutes at 40°C with 0·03 ml. portions of enzyme solution of varying strength (0·25 mgm. to 3·5 mgm. per 100 ml.) and the proteolytic activity was estimated by the modified phenol-reagent method as mentioned before. The results are presented in fig. VI.





Effect of period of hydrolysis on the protease activity:

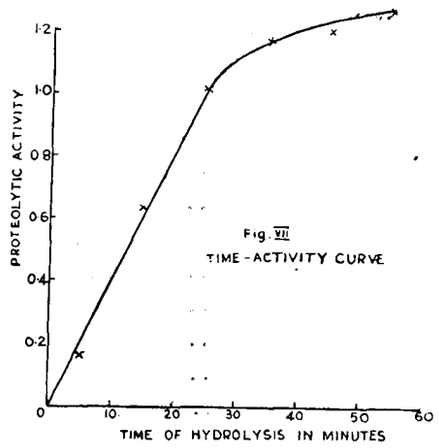
40 ml. portions of 2·5 per cent egg albumin solution in bidistilled water buffered with veronal buffer at pH 7·45 were allowed to be hydrolysed at 40°C with 0·06 ml. of enzyme solutions (2 mg/100 ml.). At regular intervals, 5 ml. of the digestion mixture were taken out and the degree of hydrolysis was estimated by the modified phenol-reagent method as mentioned before. The results are presented in fig. VII.

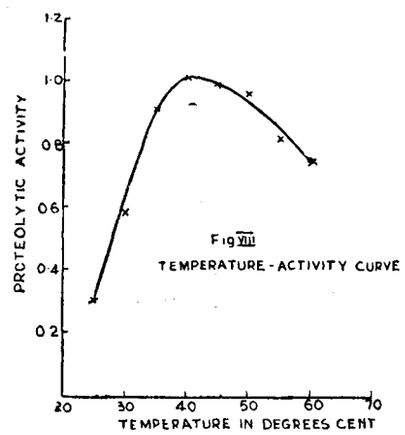
Effect of temperature on proteolytic activity:

The degree of proteolysis produced by the enzyme at different temperatures was studied. 20 ml. of 2·5 per cent egg albumin solution buffered with veronal buffer at pH 7·45 were digested for 25 minutes with 0·03 ml. enzyme solution (2 mgm./100 ml.) at different temperatures (25-60°C). The results are presented in fig. VIII.

pH stability of the protease:

In order to study the effects of pH on the stability of the protease, 5 ml. portions of the enzyme solutions (4 mgm./100 ml.) were buffered with 5 ml. portions of veronal buffer of pH ranging from 2·60-9·60. The buffered enzyme solutions were stored





at 18°C for 24 hours, the proteolytic activity was measured in the usual manner before and after storage and the loss of proteolytic activity was calculated in each case. The results are shown in table V.

TABLE V

pH stability of the protease

pH	% loss in proteolytic activity
2·60	73·27
3·60	70·30
4·65	49·50
5·30	37·62
6·10	19·80
7·25	2·97
8·10	10·89
9·60	46·53

Heat inactivation:

With a view to studying the effect of heat on the proteolytic activity, 10 ml. portions of the enzyme solution (2 mgm./100 ml.)

were heated in glass stoppered bottles at different temperatures (40-90°C) for one hour and then rapidly cooled to room temperature and the residual proteolytic activity was determined against egg albumin solution in the usual manner. The results are shown in table VI.

TABLE VI

Effect of heat on proteolytic activity

Storage temperature °C	% loss in activity
40	0
50	4·95
60	12·87
70	24·75
75	38·61
80	50·50
85	62·38
90	87·13

Effect of ultraviolet irradiation on proteolytic activity:

In order to study the effect of ultraviolet irradiation on the activity of protease, 20 ml. portions of enzyme solution (4 mgm./100 ml.) were put in different uncovered Petri dishes which were kept on a water bath maintained at 25°C. Each dish was exposed to ultraviolet rays from a mercury vapour lamp (Hanovia) in a dark room. As control, 20 ml. of the same enzyme solution were kept in another Petri dish at 25°C without exposure to light. Proteolytic activity of the enzyme solutions at the start and after regular intervals was determined as before. The liquid level in Petri dish was carefully marked and bidistilled water was added to make up the loss of water due to evaporation. The results are shown in table VII.

TABLE VII

Effect of ultraviolet irradiation on protease.

Period of irradiation in hours	% loss in proteolytic activity
3	0
6	5·94

Effect of dialysis on protease:

2 ml. portions of enzyme solution (4 mgm./100 ml.) were dialysed in several dialysis tubings against bidistilled water at refrigerator temperature (about 4°C) with frequent changes of water. At regular intervals, the dialysis tube was taken out, the contents were made upto a definite volume and the proteolytic activity was estimated against egg albumin in the usual manner. The results are presented in table VIII.

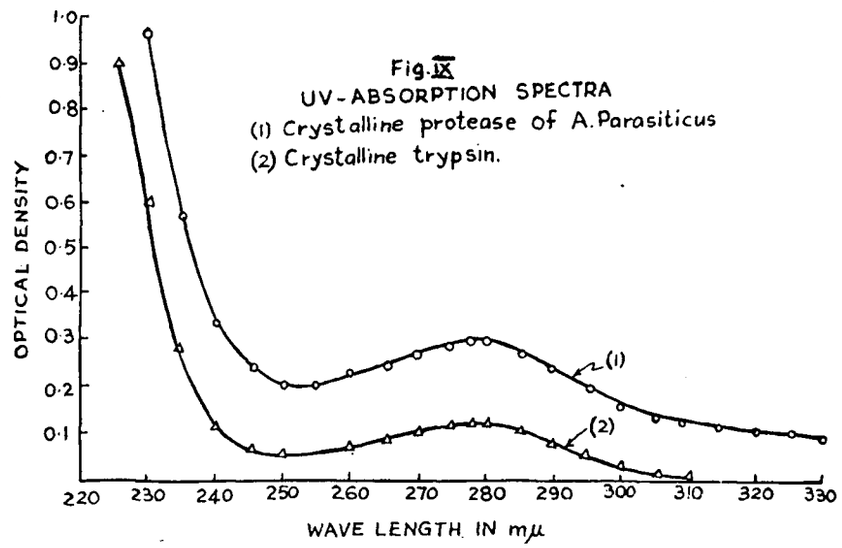
TABLE VIII

Effect on dialysis on protease.

Period of dialysis in days	% loss in proteolytic activity
3	0
5	0
7	4·95
9	4·95

Ultraviolet absorption spectrum of crystalline protease:

With a view to studying the ultraviolet absorption spectrum of the protease, the optical density of the enzyme solution (20 mgm. in 100 ml. bidistilled water) was measured at different wavelengths in a Beckman spectrophotometer (DU). In order to compare the ultraviolet absorption spectra of this enzyme and of tryp-



sin, the above experiment was repeated with twice crystallised trypsin (Nutritional Biochemical Corporation, Ohio) solution (10 mgm. in 100 ml. bidistilled water). The results are presented in Fig. IX.

Discussion

It appears from table II that the pH 7·5 at which the maximum proteolytic activity was obtained on acetone precipitation of the enzyme is the approximate isoelectric point of the protease from *A. parasiticus*. The pH has, therefore, been selected for the acetone precipitation as well as for the ammonium sulphate precipitation of this enzyme.

It was observed that the acetone precipitated enzyme when dried and dissolved in bidistilled water was not completely soluble but gave a very fine suspension. This insoluble material was removed by supercentrifuging and was found to possess very little or no proteolytic activity. The clear centrifugate obtained from supercentrifuging was found to retain the original enzyme activity. Ultrafiltration of the enzyme solution was thus found to give higher specific activity.

Table IV shows that when the original enzyme extract of the mold was subjected sequentially to the nine different treatments found to be beneficial for the purification, a highly purified protease having about 99,510-fold increase in specific activity was obtained. This highly purified protease was subsequently crystallised from veronal buffer of pH 8·4 on storing the enzyme solution at 4°C for two days.

Fig. II shows that the globular proteins which were prepared from a fresh goat skin were readily hydrolysed by the crystalline protease under suitable pH conditions; the pH range at which the maximum hydrolysis occurred was found to be 7·55 to 8·90 for albumin, 7·20 to 8·40 for globulin and 8·00 to 9·20 for mucoids. The fibrous proteins, viz., collagen, reticulin, elastin and keratin, which were prepared from skins, hides and other sources were found to be not hydrolysed by the protease under the experimental conditions. These results appear to support the views of previous workers[28, 33-36] that the action of certain proteolytic enzymes in unhairing and bating is related to the hydrolysis and removal of globular proteins of skins and hides. As collagen has been found to be not susceptible to hydrolysis by this enzyme, it may be reasonably expected that in the practical unhairing and bating with this enzyme, there may not be undue loss of hide

substance. Although the protease did not exhibit quite sharp pH optima with reference to skin albumin, globulin and mucoids, the digestibility of these proteins after urea denaturation was not studied as it was thought desirable to study the action of the enzyme on these proteins as far as possible under natural conditions.

In the case of egg albumin also sharp pH optimum was not obtained without urea denaturation (Fig. III). The figures III and IV show that the experiments on hydrolysis of the urea-denatured proteins under different pH conditions give definite pH optima and the degree of hydrolysis vary to a great extent from protein to protein. Among all the proteins examined, the digestibility of egg albumin (E. Merck) was found to be the highest. The gelatin used (Fig. V) was also found to be digestible by this enzyme. The pH optima of the enzyme with reference to the hydrolysis of egg albumin, haemoglobin, fibrin, casein and gelatin were found to be 7·45, 7·75, 8·00, 7·75 and 7·25 respectively. Wetter[17] studied the pH-activity relationship of the extracellular protease from *Mortierella renispora* and found that the enzyme had broad pH optimum (6·5-10·5) on haemoglobin with peak activity at pH 7·5. Johnson[13] reported that the pH optimum of the proteinase from *A. parasiticus* was 7·3 with reference to gelatin and casein. McConnell[16] found that the pH optimum of the proteases from species *Alternaria, Streptomyces, Mortierella* and *Gliocladium* against gelatin was 7·0.

Fig. VI indicates that with varying enzyme concentration and constant substrate concentration, the activity is varied. The opimum concentration of the enzyme for the hydrolysis of 0·5 gm. egg albumin was found to be 0·6 µg. upto which the linear relationship between enzyme concentration and activity was obtained.

Fig. VII shows that the optimum period of hydrolysis of 0·5 gm. egg albumin by 0·6 µg. of the enzyme was 25 minutes. Hence 252 minutes digestion period was used throughout the present investigation.

Fig. VIII shows that the maximum digestion of egg albumin by the protease under the experimental conditions, occurred at 40°C. Although rate of reaction of the enzyme increases with increase in temperature till it reaches the optimum, two independent processes, viz., the catalysed reaction and the thermal inactivation of the enzyme may simultaneously occur because of the increase in temperature. At temperatures lower than the opti-

mum the catalysed reaction is mainly influenced by temperature while at temperatures higher than the optimum the heat inactivation of the enzyme chiefly progresses.

It may be seen from table V that the stability of the enzyme was greater at pH 6·10 to 8·10, the maximum stability being at 7·25. Highly acidic or highly alkaline pH conditions were found to reduce the stability of the enzyme appreciably. Johnson[13] observed that a crude preparation of the proteinase from *A. parasiticus* was unstable to acid and alkali, losing 95% of its activity at pH 3·5 and 80% at pH 9·5 in 14 hours at room temperature. Wetter[17] observed that the extracellular proteinase from *Mortierella renispora* was stable at 1° and 25°C from pH 4·9 to 9·5.

Table VI indicates that the critical inactivation temperature for the protease, i.e., the temperature at which about half the enzyme activity was destroyed in one hour, was 80°C. The enzyme was susceptible to inactivation at higher temperatures. It was found to be completely inactivated within one hour when stored at 95°C. Northrop[37] reported that the thermal inactivation of certain crystalline proteases, viz., trypsin, chymotrypsin and pepsinogen was reversible on cooling. In the present study, it was found that the heat inactivation of the crystalline protease was partially reversible on cooling.

It may be seen from table VII that there was no appreciable reduction of the protease activity on exposure to ultraviolet rays at 25°C for a period of 6 hours.

It may be seen from table VIII that there was no appreciable reduction of protease actiivity on dialysis for a period of nine days against bidistilled water at about 4°C.

Fig. IX shows that the ultraviolet absorption by the protease at different wavelengths was similar to that of the crystalline trypsin. It was observed that the maximum ultraviolet absorption was at 2800 A and the minimum at 2500 A. In view of this observation and also on the basis of the results obtained with regard to pH-activity relationship and other properties of this protease, it appears that this enzyme is more or less of the tryptic type.

Acknowledgment

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

REFERENCES

1. Kocholaty, W. and Krejci, L. E., *Arch. Biochem.* **18**, 1 (1948).
2. Debellis, R., Mandl, I., Maclennan, J. D. and Howes, E. L., *Nature* **174**, 1191, 4443 (1954).
3. Bidwell, E., *Biochem. J.* **46**, 589 (1950).
4. Bensusan, H. B., Derow, M. A. and Walker, B. S., *Arch. Biochem, Biophys.* **49**, 293 (1954).
5. Emanuiloff, I., *Enzymologia*, **20**, 173 (1959).
6. Elliott, S. D., *J. Exp. Med.* **92**, 201 (1950).
7. Fuku-moto, J. and Negoro, H., *Proc. Japan Acad.* **27**, 441 (1951).
8. Guntelberg, A. V. and Ottesen, M., *Nature* **170**, 802 (1952).
9. Hagihara, B., *Ann. Rept. Sci. Works, Fac. Sci., Osaka Univ.* **2**, 35 (1954).
10. Hagihara, B., Matsubara, H., Nakai, M. and Okunuki, K., *J. Biochem. (Japan)* **45**, 185 (1958).
11. Crewther, W. G. and Lennox, F. G., *Nature*, **165**, 680 (1950).
12. Miyake, S., Yoshimura, S. and Yagishita, K., *Agr. Ser. Agr. Chem. Ed.* **1**, 47 (1954).
13. Johnson, M. J., *Z. Physiol. Chem.* **224**, 163 (1934).
14. Matsubara, H., Hagihara, B., Nakai, M., Komaki, T., Yonetani, T. and Okunuki, K., *J. Biochem. (Japan)* **45**, 251 (1958).
15. Viswanatha, T. and Liener, I.E., *Arch. Biochem. Biophys.* **61**, 410 (1956).
16. McConnell, W. B., *Can. J. Research*, **28C**, 600 (1950).
17. Wetter, L. R., *Can. J. Botany* **30**, 685 (1952).
18. *Idem. Can. J. Biochem. Physiol.* **32**, 60 (1954).
19. Dhar, S. C. and Bose, S. M., *Bull. Cent. Leath. Res. Inst.* **6**, 69 (1959),
20. *Idem., Ibid.* **6**, 13 (1959).
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
22. Bose, S. M. and Madhavakrishna, W., *Bull. Cent. Leath. Res. Inst.* **3**, 329 (1957).
23. Bose, S. M., Dhar, S. C. and Das, B. M., *Tanner* **11**, Nos. 2 & 3, (1956):
24. Warburg, O. and Christian, W., *Biochem. Z.* **310**, 384 (1941-42).
25. Keilin, D. and Hartree, E. F., *Biochem. J.* **49**, 88 (1951).
26. Durrum, E. L., *J. Am. Chem. Soc.* **72**, 2943 (1950).
27. Joseph, K. T. and Bose, S. M., *Bull. Cent. Leath. Res. Inst.* **4**, 343 (1958):
28. Bose, S. M., Madhavakrishna, W. and Das, B. M., *J. Am. Leather Chemists' Assoc.* **50**, 192 (1955).
29. Bose, S. M. and Madhavakrishna, W., *Enzymologia* **19**, 186 (1958).
30. Northrop, J. H., *J. Gen. Physiol.* **16**, 41 (1932).
31. Anson, M. L., *Ibid.* **22**, 79 (1938).
32. Krishnamurthi, C. R. and Subrahmanian, V., *Ind. J. Dairy Sci.* **2**, 19 (1949).
33. Schneider, F., *Collegium* No. 809, 522 (1937).
34. Roddy, W. T., *J. Am. Leather Chemists' Assoc.* **31**, 410 (1942).
35. Kritzinger, C. C., *Ibid.* **46**, 350 (1951).
36. Burton, D., Reed, R. and Flint, F.O., *J. Soc. Leather Trades' Chemists* **37**, 82 (1953).
37. Northrop, J. H., "Crystalline enzymes".

RESEARCH BEARS FRUIT

Manufacture of chrome retan leather*

BY

A. GANESAN, S. P. GHOSH & S. M. DE

Introduction

Three different processes for manufacture of chrome retan leather were demonstrated recently at Calcutta. The first of these is described below:

PROCESS NO. I

Raw Materials:

8 wet salted cow hides (155 lb., i.e., 70·30 kg.) from Raja Bazar Market, Calcutta, generally used to make cheap chrome leathers were taken.

Soaking:

The hides were soaked for 3 hours in a pit containing plain water sufficient to immerse the goods, with the addition of 2 gallons (9·09 litres) fresh lime liquor. Soaked weight was 180 lb. (81·65 kg.).

Liming:

The hides were limed in a pit with the following:

2% sodium sulphide (60-62%), on soaked weight.
1% ammonium sulphate, -do-
300% water, -do-
3% slaked lime, -do-

The sodium sulphide and ammonium sulphate were first dissolved in the requisite amount of water in a pit. The hides were handled in this bath for 10 minutes and then taken out. Lime was then stirred in. The goods were put back in the liquor, handled and left overnight.

*Process demonstrated during the period December 1960 and January 1961, in Messrs. Hindusthan Tannery, Dhapa, Calcutta.

2nd day: The hides were handled thrice.

3rd day: The limed pelts were given a wash in plain water, cut into sides and hand fleshed. The fleshed weight was 185 lb. (83·91 kg.).

Deliming:

The fleshed pelts were taken straight for deliming in a drum containing ½% ammonium sulphate and 200% water. The drum was run for ½ hour by which time, one-third of the total thickness was delimed; the pelt was washed once in plain water.

Pickling:

Pickling was done with the following chemicals:
2% sulphuric acid (sp. gr. 1·73)
5% common salt.
100% water.

The hides were drummed in the pickle bath for 2 hours and left well immersed overnight.

Preparation of stock chrome liquor:

Chrome liquor was prepared using the following:

Sodium bichromate	..	28 lb. (12·70 kg.)
Sulphuric acid (60°Bé)	..	28 „ (-do-)
Brown sugar	..	7 „ (3·17 kg.)
Water	..	56 „ (25·40 kg.)

The stock liquor* was made upto 125 lbs. (56·69 kg.).

4th day: The pH of the exhaust pickle bath was 2·5. Two-thirds of the pickle liquor was drained and an equal quantity of fresh water was added into the drum; the pickled pelts were drummed with the addition of 35 lb. (15·88 kg.) of prepared stock chrome liquor (3·5% bichromate equivalent on fleshed weight) for 5 hours. 1·0% sodium bicarbonate, made into a 10% solution, was added over a period of 1 hour. The drum was run for ½ hour after the last addition of sodium bicarbonate. The pH of the exhaust chrome bath was 3·7. The sides were piled overnight.

5*th day*: The sides were sammed, shaved to 1·5 mm. The shaved weight was 80 lb. (32·29 kg.). (No splitting machine was available).

Rechroming:

The shaved sides were put in a dry drum and rechromed for 1 hour in the drum with 6% (on shaved weight) chrome extract

* The dichromate was completely reduced by addition of 2 lb. (0·906 kg.) sodium bisulphite.

powder (B & C make) along with the addition of 9 oz.% sodium bicarbonate in 35% float. The chrome was completely absorbed by the hides. The pH of the leather was 4·0. The leathers were then piled.

6th day: The leathers were drummed for ½ hour with 12 oz. % sodium bicarbonate in two instalments in 200% float and left overnight in the neutralising bath.

7th day: Next morning, the drum was run for 5 minutes. The cut section had uniformly a pH of about 4·5.

Fatliquoring:

The goods were washed for 5 minutes in 200% water and the fatliquor of the following composition was used:

2·0% sulphated pungam oil.
2·0% free fish oil.
100% water.
Fat liquoring was carried out for 45 minutes in cold water.

Retanning:

The tan liquor prepared with the following was used for retanning:

8% cutch extract No. II.
1% kenmosa (mimosa extract) spray dried extract.
50% total float including water used for dissolving the extract.

Retanning was started with the addition of the tan liquor in three instalments at intervals of 20 minutes. 5·0% common salt was added, 30 minutes after the last instalment, to get better exhaustion of tannins. The drum was run for another ½ hour. The total period of drumming was 2 hours. The retanned goods were then given three washes for 5 minutes each in 200% float.

Refatliquoring:

The leathers were refatliquored with a mixture of the following:

2½% sulphated fish oil.
½% raw fish oil.
¼% tallow.
¼% kid oil.
¾% kerosene.
Total float 50%.
The goods were drummed for 10 minutes in cold water.

8th day: The goods were sammed by hooking and straight-away nailed on board in the sun to make the leather flat (Temp. 80°F or 15°C, R.H. 60% approx). The leathers were dried to about 40% moisture content, removed from the board and hung up in shade for complete drying. The leathers thus obtained were free of wrinkles and were quite flat.

9th day: The leathers were conditioned with saw dust to have about 30% moisture.

10th day: The leathers were staked on the edges and butts only and shaved to 1·5 mm. all over the hide.

11th day: The goods were trimmed, buffed and snuffed on the grain with 220 grit emery paper. These were then lightly staked in a modified staking machine where necessary.

Finishing:

A sealer coat of the following composition was applied: 2 parts of 10% linseed mucilage, 1 part of soft soap solution, the pH of which was adjusted to 6·0 by acetic acid.

One brush coat of the following season was then given:

2 lb. (0·907 kg.) pigment paste (Handymen Industries Ltd., Madras).
3 lb. (1·360 kg.) F.C.B.O. (B.A.S.F.).
8 oz. (0·227 kg.) 10% casein solution.
4 oz. (0·113 kg.) 10% linseed mucilage.
2 oz. (0·56 kg.) soft soap neutralised to 5·6 by acetic acid.
Water to make 6 litres.

The second and third brush coats were applied with the addition of 170 cc. of formaldehyde per gallon of season. A top spray of the following composition was given:

10% casein solution	500 cc.	A
water	1000 cc.	A
40% formaldehyde	250 cc.	B
water	250 cc.	B

A and B were mixed together and used. In the absence of a spraying booth, the above was applied by brush once and the leathers dried. The leathers were then measured and hair-cell plated.

Measurement: 198 sq. ft.

A final top gloss coat of an alkyd resin was given to improve lustre and waterproofness.

Our thanks are due to M/s Hindustan Tannery, for providing the necessary facilities.

TECHNICAL SEMINAR

Instruments and analytical techniques in leather research*

BY

S. ANANTHANARAYANAN

A complexometric titration technique developed for the estimation of Cr^{3+} [*Bull. C.L.R.I.* **6**, 595 (1960)] was found to hold good for the estimation of Cr^{3+} in basic chrome liquors containing masking salts, organic reduced chrome liquors and even in the presence of Cr (VI) with an accuracy of 1·1%.

A study on the violet chrome—EDTA complex in the U-V range indicated that it had no characteristic maximum. A photometric titration for the estimation of Cr^{3+} in the U-V region has been proposed. Spectrophotometric studies on chrome-pyridine and chrome-urotropine complexes in the visible region indicated that urotropine had lesser tendency than pyridine to enter into the coordination sphere of the chrome complex. The studies in the U-V and visible range on molybdate-vegetable tannin solutions indicated the formation of complexes between anionic molybdate and phenolic groups in favourable position of the vegetable tannin compounds.

A nonaqueous titration technique using ethylene glycol and isopropanol mixture and sodium borate has been developed for the differential estimation of protein bound and chrome bound acids. Comparative studies using the existing methods indicated that all of them failed to giive satisfactory results due to hydrolysis and replacement reactions. An improvement over the existing pyridine method by substituting 4% solution of urotropine to differentiate ionically held and complexly held sulphato groups in the chrome tanned leather has been made. An attempt has been made utilising the data obtained from nonaqueous titration technique to find out qualitatively the ionically held sulphato groups attached to chrome complex and thereby the mode of fixation of cationic chrome complexes, assuming that chrome complex fixed on the hide protein as purely cationic.

Studies on the precipitate obtained on addition of EDTA to 33·3% basic chrome liquor (1:1 and 1:2 mole basis) indicated that the added EDTA did not replace olated OH groups, although the sulphato groups were completely replaced by the ligands of EDTA [*Bull. C.L.R.I.* **6**, 595 (1960]. The precipitate formation at low pH value substantiated with the data obtained by infra-red, and potentiometric titration and micro-analysis of the precipitate for carbon and chromium contents indicated that the precipitate might not be a mere chromium hydroxide in which EDTA was physically adsorbed. The anamolous behaviour of EDTA as bridge forming agent contributing to aggregation of precipitate was explained by assuming the coordination of N of complexed EDTA with olated hydroxyl groups through hydrogen bonds.

From studies on treatment of chrome tanned leather with EDTA, molybdate and dihydrogen phosphate, it was concluded that (i) the cationic complex changed into non-cationic one lowering the I.E.P. and hydrothermal stability, (ii) sulphato groups were replaced by EDTA and phosphate, (iii) olated OH groups were affected in phosphate and not in molybdate and EDTA treatment and (iv) stripping of Cr was observed in case of EDTA which pointed out disturbance of collagen carboxyl groups involved in crosslinking to different extents based upon total fixed chrome content.

With vegetable tannins, retannage studies of EDTA molybdate pretreatment chromed hide powder indicated that the fixation of vegetable tannins was governed by the charge of the chrome complex, nature of the complex and available sites in the protein substrate. A study on molybdate prechromed and retanned with vegetable tannin indicated that the increase in fixed organic matter led to an increase in crosslinks formation due to complex forming tendency of molybdate with phenolic OH groups present in the vegetable tannins in favourable positions. A study on pretreatment with molybdate before retannage was found to differ with the type of tanning material.

*Summary of the talk given on January 6, 1961.

TECHNICAL SEMINAR

Studies on oil tannage*

BY

K. S. JAYARAMAN

The oxidisability of fish oil was investigated using Mackey test. It was observed that the oxidisability was governed by the iodine value of the oil and not by the acid value. A knowledge of the Mackey value of the oil in relation to iodine value and tanning trials indicated that oils having iodine value less than 125·0 did not possess any tanning action.

Oxidation studies were carried out on fish oil by blowing air at different temperatures; effect of factors like moisture and surface area was also investigated. The studies indicated that the following different reactions took place simultaneously; (1) formation of peroxides and hydroperoxides, (2) polymerisation to products causing an increase in density and (3) decomposition of peroxides into aldehydes. The extent to which these reactions take place is governed by the various factors mentioned earlier.

An investigation on the effect of concentration, moisture and time on tanning with fish oil indicated the following: (1) atmospheric moisture was adequate to effect tanning action, larger amounts of moisture causing only slightly higher fixation of oil, (2) peroxides and fatty acids were formed to a greater extent when larger amounts of oil were used but the fixation of oil did not follow the same trend and (3) the peroxides and hydroperoxides were fixed at the nonionic protein groups and the aldehydes formed as a result of the decomposition of peroxides, at the basic protein groups.

A study on the effect of alkali wash for removing the unreacted oil indicated a simultaneous fat-liquoring action being exerted during such a treatment, imparting the desirable characteristics to chamois leather.

*Summary of talk given on January 20, 1961.

TECHNICAL SEMINAR

Histological study of Indian cattle and buffalo hides*

BY

S. K. SARKAR

The nature and distribution of the various hide tissues which determine the leather making property vary not only from hide to hide but also over the area of the same hide. The sex and the place of origin of the animal have also some influence on the hide tissues. Although built up on the same structural plan, cattle hides are fundamentally different from buffalo hides. Indian cattle and buffalo hides have some inherent peculiarities of their own which are likely to be reflected in the leathers made from them. Buffalo hides, in general, are thicker than the cattle hides of equal weight but by nature are less compact and have lower angle of weave. Indian cattle and buffalo hides may be utilised with advantage for the production of the different types of leather for which they are individually better suited. Cattle hides with smooth and tight grain surface and thicker grain layer are suitable for the production of shoe upper leather; the rough grain surface and thicker corium make buffalo hides unsuitable for this purpose. Female cattle hides with tighter and finer grain surface appear to be more suitable for upper leathers than those from male animals; the latter because of the thinner grain layer and thicker corium with bigger fibre bundles may be better suited for the manufacture of harness, upholstery, bag and case leather or where suppleness and pliability are not of primary consideration. The quality of thickness, thinner grain layer and thicker corium with low angle of weave make buffalo hides suitable for the manufacture of mechanical leather, such as belting, picking band and pickers of power loom. She-buffalo hide appears to be better suited for this purpose than the he-buffalo hide. Because of the low angle of weave, buffalo hide may not be suitable for the production of sole leather; and if this hide is to be used for sole leather, it has to be filled heavily to make the texture compact. Indian buffalo hides with required thickness may be quite suitable for the manufacture of industrial heavy leathers; but as Indian hides are not uniform in texture over their area, a uniform leather cannot be expected.

*Summary of talk given on January 27, 1961.

CLASSIFIED ABSTRACTS

CHROME TANNING

The problem of loose grain in chrome upper leathers.

W. Rieger, *Tanner* **15**, No. 2, 53-60 (1960). After observing that loose grain is often due to the nature of the hides themselves, the author describes and discusses the various operations involved in the processing of box sides, with a view to achieve maximum tightness in the finished leather without however impairing other favourable factors like fullness, feel, fineness of grain and so on.

Too short a soak causes variations in grain tightness, and a prolonged soak in summer gives rise to poor flanks. A duration of 24 hours for calfskins and 36-48 hours for sides, in cold water at a temperature of 16-18°C is recommended. Mechanical action should be gentle, and employed only sparingly. Breaking on the beam is advisable, unless the skins are of the light weight type.

Lime painting is preferred as it helps to achieve smoothness of grain, increased yield in area and growth marks and drawn grain are less apparent in the finished leather. Sammying before painting should be done. Reliming for box sides is done with 3·0-4·5% sodium sulphide conc. and 2-4% slaked lime for 18-24 hours. For calfskins, the period may be greater, e.g. 36-48 hours. The temperature of the lime-liquor should be 21-25°C and mechanical movement should be done gently and sparingly. Splitting in the limed stage is not advisable, as it leads to looseness in the finished leather. It is best done after tanning.

Deliming and bating are interrelated processes and are in turn related to liming and pickling. A preliminary wash for 10-25 minutes, starting with a temperature of 25°C and raising it to 33°C, followed by deliming for 15-30 minutes, using a customary deliming agent (cut from butt should be ¼ acidic to phenolphthalein from grain and flesh) and bating for 20-45 minutes using 0·4-0·7% of a pancreatic bate are the recommended processes. This series of operations are so controlled that at the end of the bating, the centre of pelt still reacts alkaline to phenolphthalein. A short rinse at this stage (10-20 minutes) helps to

remove the lime salts. Stress is laid on the conclusion that the effect of bate on the tightness or looseness of the ultimate product is really determined by the extent of deliming.

In pickling, the amount and type of neutral salt are important considerations. Partial replacement of the chloride with sulphate or formate, leads to a tighter break in the leather and a more uniform tannage particularly when the latter is used. As in other pretanning operations, period of drumming should be cut down to the minimum, if necessary, in favour of the overnight method of pickling. Addition of small quantities of chrome alum, formaldehyde, in the pickle after about 1 hour's drumming, helps to fix the grain and thus achieve tightness in the final product.

In tannng, it is better to use previously basified chrome liquor compared to the technique of basification in the bath itself, as the latter method leads to forced grain, overthick grain layer and uneven fixation of chrome. As mellow leather is in demand, the tannage is usually finished at high pH values. Use of formate in tanning helps to avoid the bad characteristics of tanning at high pH, like looseness, coarse grain etc. A short float (50-70%) and warming up of the bath (28-35°C) towards the end of tanning help to fix greater amount of Cr_2O_3 in leather. Fat liquors resistant to salt and acid, when used during tanning, though effective in producing mellow leather may cause trouble in subsequent operations leading to looseness, nonlevel dyeing etc. The method of tanning in two stages using half the quantity of chrome at low basicity (33%) and finishing it with chrome of 50% basicity, after doing the levelling, sorting, etc. for retannage, white tannage and so on, at the intermediate stage, is recommended for its obvious advantages.

A technique developed by the Fabriken Bayer West Germany, is of particular interest. In this method, the chrome extract (Chromosols) can be added straight to the drum in the form of a powder, without prior dissolution in water. The pH value of the exhaust chrome bath is another important factor that influences chrome tanning and it should not exceed 4·00 if the grain is to be smooth. The leather should be completely resistant to boiling water when removed from the drum and aged at least for two days on flat boards rather than on steep horses.

All the factors described above, contribute in their own small way, towards a tighter leather.

P. S. V.

TESTING

A rapid method of total lipid extraction and purification.

E. G. Bligh & W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959). Lipid decomposition studies in frozen fish have led to the development of a simple and rapid method for the extraction and purification of lipids from biological materials. The entire procedure can be carried out in approximately 10 minutes; it is efficient, reproducible, and free from deleterious manipulations. The wet tissue is homogenised with a mixture of chloroform and methanol in such proportions that a miscible system is formed with the water in the tissue. Dilution with chloroform and water separates the homogenate ino two layers, the chloroform layer containing all the lipids and the methanolic layer containing all the non-lipid extract is obtained merely by isolating the chloroform layer. The method has been applied to fish muscle and may easily be adapted to use with other tissues.

A micromethod for the determination of sulphate by flame photometry.

G. A. Robinson, *Can. J. Biochem. Physiol.* 38, 643 (1960). A micromethod for the determination of 0·1 to 3·5 μg of sulphate is described. Acidified barium chloride solution is added to samples containing free and esterified sulphate, and the resultant mixtures are dried at 95°C. Soluble materials are redissolved with a 90% solution of acidified ethanol, 0·1*N* for hydrochloric acid, containing 250 μg lithium per milliliter. After addition of the ethanol solution approximately eight hours are required for the establishment of equilibrium (as indicated by a radiosulphur label) between solid and dissolved barium sulphate. Barium present in solution is then determined quantitatively by flame photometry, with the ethanol-lithium medium increasing the primary emission of the barium by 15 times. The sulphate content of the sample is read from a standard curve. Estimations on microgram quantities of some organic and inorganic sulphur-containing compounds are given.

Paper chromatography of complex biological materials by solvent redevelopment without prior removal of salts or proteins.

Arthur E. Pasieka, *Can. J. Biochem. Physiol.* 38, 1137 (1960). A solvent redeveloping technique has been devised by which amino acids, peptides, and sugars can be separated from complex mixtures in the presence of high concentrations of salts and proteins. The saparations are effected by two to four successive 18-hour solvent developments witn drying between each 18-hour period before subsequent staining of the chromatograms. Better separations and resolutions are obtained by such successive 18-hour solvent developments than by one continuous solvent development for an equivalent time. The effect of these redevelopments on the separations and resolutions of biological compounds is illustrated at various stages by photographs of one- and two-dimensional chromatograms. The redevelopment technique requires filter paper sheets upto 4 ft. in length for one-dimensional analytical and preparative types of chromatograms.

Measurement of half-wave potentials with cylindrical electrodes.

Evan Morgan, J. E. Harrar and A. L. Crittenden (Depart. of Chem., Univ. of Washington, Seattle 5, Wash.) *Anal. Chem.* **32**, 756 (1960). Cylindrical microelectrodes have been useful in voltammetry for the measurement of oxidation potentials where mass transfer is diffusion-controlled. Current-potential relationships are only slightly different at small times of electrolysis.

Spectrophotometric determination of carboxyl in oxidized starch.

Herbert C. Cheung, Benjamin Carroll and C. Edwin Weill, (Chem. Depart., Rutgers, The State Univ., Newark, N.J.) *Anal. Chem.* **32**, 818 (1960). Methylene blue is used in a spectrophotometric procedure to determine the carboxyl content of oxidized starches produced by the hypochlorite oxidation process. The basis for the method is the apparent constancy of the binding affinity of methylene blue and carboxylate ion at infinite dilution of the dye. Prior removal of inorganic cations usually present in carboxylated starches is not essential. For the special case of samples with exceedingly high carboxyl content, a simpler and more direct colorimetric procedure may be used.

Amperometric determination of acetaldehyde with hydroxylamine hydrochloride.

R. E. Van Atta, W. W. Harrison and D. E. Sellers (Depart. of Chem., Southern Illinois Univ., Carbondale, Ill.) *Anal. Chem.* **32**, 1548 (1960). The oxime produced by the reaction of acetaldehyde with hydroxylamine hydrochloride yields a polarographic reduction wave ideally suited for the amperometric titration of the aldehyde in aqueous solutions.. The polarographic current is measured at a constant potential of -1·25 volts *vs.* the saturated calomel electrode, using a dropping mercury electrode. The method is convenient and rapid, requiring no more than 10 minutes for

completion after initial preparation of the test solution. Aqueous solutions of CH_3CHO in the range of 1 to 100mM may be titrated with 0·1M reagent with an average accuracy of ± 1% of the amount of acetaldehyde taken; the concentration range may be extended by increasing or decreasing the concentration of the titrant. Impurities, such as ethyl alcohol, ethyl acetate, or acetic acid, do not interfere in concentrations as great as five times the concentration. The technique has been applied successfully to the analysis of commercial acetaldehyde preparations.

Estimation of hydroxyl, methyl, and phenyl in dimethyldiphenyl silicone resins by infrared spectrophotometry.

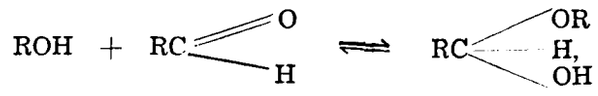
E. R. Shull (Res. Lab., Linde Co., Div. of Union Carbide Corp., Tonawanda, N. Y.) *Anal. Chem.* **32**, 1627 (1960). An infra-red spectrophotometric method of estimating hydroxyl, methyl, and phenyl in dimethyldiphenyl silicone resins is described. The analysis is calculated from scans over the 2- to 4- micron (μ) region. The 2·8 "free" hydroxyl, 2·9 "hydrogen-bonded" hydroxyl, 3·3 phenyl, and 3·4-micron methyl bands are used. Absorbance ratios are employed to make the analysis independent of variations in sample thickness. The methyl (or phenyl) group is used as an internal standard in the hydroxyl content calculations. The method provides for the determination of hydroxyl as free and hydrogen-bonded hydroxyl; total hydroxyl is obtained as the sum of these two types. The results are good to about 10% (relative). This is the only known method of determining free and hydrogen-bonded hydroxyl in silicone resins.

Some physical characteristics of the 2, 4-dinitrophenylhydrazine and semicarbazide derivatives of three-carbon carbonyls.

J. C. Underwood and Harry G. Lento (Eastern Reg. Res. Lab., Eastern Utilization Res. and Development Div., Agric. Res. Service, U. S. Depart of Agric., Philadelphia 18, Pa) *Anal. Chem.* **32**, 1656 (1960). A means of identifying the short-chain hydroxyaldehydes and ketones resulting from the alkaline degradation of hexoses was needed, and the literature was found lacking in data which permit the differentiation of these compounds (acetol, dihydroxyacetone, glyceraldehyde, hydroxypyruvaldehyde, and methylglyoxal). The 2, 4-dinitrophenylhydrazine and semicarbazide derivatives of these trioses were therefore prepared, purified, and characterized by the determination of their elemental composition, melting points, and ultraviolet and infrared spectral absorptions.

Determination of aldehydes from hemiacetal formation.

J. S. Forrester (Esso Res. Labs., Esso Standard, Humble Oil & Refining Co., Baton Rouge, La.) *Anal. Chem.* **32**, 1668 (1960). The temperature-sensitive equilibrium reaction between aldehydes and alcohols,



can serve as a useful analytical tool. Detection of carbonyl compounds in complex mixtures is possible by comparing the ultraviolet spectrum of the mixture of two different temperatures. The technique is particularly suitable for continuous process analysis.

Primary standards for titration of anionic detergents using quaternary ammonium halides.

Benjamin Veldhuis (General Chemical Division, Allied Chemical Corp., Morristown, N. J.) *Anal. Chem.* **32**, 1681 (1960). The alkaline salts of polyhalogenated benzenesulfonic acids have been found suitable for direct standardization of quaternary ammonium halides. These stable sulfonates can be prepared easily.

Optimum conditions for separation in gas chromatography.

J. Calvin Giddings (Depart. of Chem. Univ. of Utah, Salt Lake City 12, Utah) *Anal. Chem.* **32**, 1707 (1960). A procedure is outlined in which the separation function (a quantitative measure of the extent of separation) can be mathematically optimized with respect to the variables that influence the chromatogram. By using a simplified plate height expression for packed columns, optimum column length, flow velocity, temperature, particle diameter, and pressure are either derived or discussed. The optimum values of flow velocity, pressure, tube diameter, temperature, and thickness of the liquid layer are derived for capillary columns. The method may be extended to other variables and techniques. The problem of minimizing the analysis time is discussed, and a method of obtaining the variables for this is presented.

Gas chromatographic characterization of fatty acids. Identification constants for mono- and dicarboxylic methyl esters.

Thomas K. Miwa, Kenneth L. Mikolajczak, Fontaine R. Earle and Ivan A. Wolff (Northern Utilization Res. and Development Div., Agric. Res. Service, U.S. Depart. of Agric., 1815 North Univ., Peoria, Ill.) *Anal. Chem.* **32**, 1739 (1960). Readily reproducible

numerical constants were determined for characterizing mono- and dicarboxylic methyl esters by gas-liquid chromatography. For a specified column packing and carrier gas, these constants, called equivalent chain lengths, are independent of experimental conditions. A combination of two values, obtaned by use of polar and nonpolar packing, is sufficient to characterize most fatty acids.

Use of a conventional mass spectrometer as a detector for gas chromatography.

L. P. Lindeman and J. L. Annis (California Res. Corp., Richmond Calif.) *Anal. Chem.* **32**, 1742 (1960). Conventional magnetic field mass spectrometers can readily be used as auxiliary gas chromatography detectors in a directly coupled arrangement. Such a system has advantages in the identification of multicomponent chromatographic peaks and of components appearing as shoulders or in the background over trapping procedures. The procedure also gives a good quantitative breakdown of multicomponent peaks. This is illustrated wth known mixures and a previously analyzed reformer charge stock.

Rapid gas chromatographic method for determination of carbon and hydrogen.

Alfred M. Vogel and Joseph J. Quattrone, (Chem. Depart., Adelphi College, Garden City, N. Y.) *Anal. Chem.* **32**, 1754 (1960). A rapid, easy method for the determination of carbon and hydrogen in organic compounds involves a bomb combustion of an 8- to 11-mg. sample in an oxygen atmosphere, sampling the products of combustion, subjecting the sample to gas chromatography, and evaluating carbon and hydrogen in terms of the planimeter-measured integrated areas for water vapour and carbon dioxide. Based on the results obtained from five organic compounds, the average deviation from the known values was 5·0 parts per thousand for carbon and 8·4 for hydrogen. The total time for a single run is 17 minutes. Triplicate results from a single combustion can be obtained in about 40 mnutes.

Ion exchange chromatography of amino acids. Study of effects of high pressures and fast flow rates.

Paul B. Hamilton (Alfred I. du Pont Institute of the Nemours Foundation, Wilmington 99, Del.) *Anal. Chem.* **32**, 1779 (1960). Glass columns and pump connections to them were designed to tolerate fluid pressures in excess of 600 p.s.i. Linear flow rates upto 0·17 cm. sec. ^{1} through the columns packed with 2·4, 4·6, or 8·0 x 10^{-3} cm. diameter resin particles were used. Pressure as

a function of particle diameter, column length, flow rate, and temperature were studied. The effect of flow rate and particle diameter on the resolution of some amino acids is indicated. Multiple section columns were made and tested.

Ion exchange chromatography of amino acids. Analysis of diffusion (Mass transfer) mechanisms.

Paul B. Hamilton, Donald C. Bogue and Roberta A. Anderson (Alfred I. du Pont Institue, Wilmington, Del., and Depart. of Chem. Engg., Univ. of Delaware, Newark, Del.) *Anal. Chem.* **32**, 1782 (1960). In this study, a theoretical approach to the ion exchange chromatography of amino acids was followed. Equations were derived which explicitly relate the current "plate" and "rate" theories and which combine the key phenomena and the column operating variables in compact algebraic form. The experimental results from the chromatography of cysteic acid, taurine, aspartic acid, serine, glutamic acid, glycine, alanine, glucosamine, and valine on columns packed with spherical beads of sulfonated polystyrene-divinylbenzene cationic exchange resin were used to test the equations. Resin particle diameter, column diameter, column length, flow rate, temperature, pH, and sodium ion concentration were studied as variables. The role of mass transfer phenomena (solid, liquid, and axial diffusion) was examined. Tentative numerical values of coefficients of solid diffusion, D_s, for some of the solutes in the resin were obtained. The resolution of two components was expressed by a simple algebraical formula which could be developed to show clearly the fundamental phenomena upon which resolution depends.

THEORIES

Proteins in fish muscle. 16. Adenosinetriphosphatase activity of cod myosin and actomysin.

J. R. Dingle & J. A. Hines, *Can. J. Biochem. Physiol.* **38**, 1437 (1960). The adenosinetriphosphatase (ATPase) activities of actomyosin extracts of prerigor and postrigor cod muscle, and of myosins prepared from them by ultra-centrifugation in the presence ATP, have been measured at ionic concentration 0·1 and pH 7·3. The actomyosin ATPases were strongly activated by Mg^{++}, in agreement with corresponding rabbit skeletal muscle proteins. Ca^{++} ion also moderately activated the actomyosin ATPases, but had little effect on the myosin ATPases. When actomyosin was precipitated by dilution in the presence of ATP and Mg^{++}, the

characteristic activation by Mg^{++} was lost. The myosin ATPases were very labile.

Biochemical effects of irradiation on liver regeneration.

D. K. Myers, *Can. J. Biochem. Physiol.*, **38**, 1059 (1960). Little change in the total weight, number of cell nuclei, and amounts of protein, ribonucleic acid, and deoxyribonucleic acid occurs in rat livers after local irradiation of the liver region. However, irradiation of the normal liver before partial hepatectomy does retard subsequent DNA accumulation and increase in number of cell nuclei. These effects appear to be due to a localised action of the radiation on the liver cells. The lag in DNA accumulation is not accompanied by a marked accumulation of acid-soluble purine-deoxyribose derivatives.

Biosynthesis of quercetin in buckwheat. Part III.

J. E. Watkin & A. C. Neish, *Can. J. Biochem. Physiol*, **38**, 559 (1960). The effect on the conversion of carbon^{14} to quercetin by a buckwheat plant caused by variation in the dose rate of a carbon^{14}-labelled precursor in μ moles over a 100-fold range has been studied. The results expressed as dilution of carbon^{14} varied over a 200-fold range for the same precursor with most of the variation being found at low dose rates. When expressed as the percentage of administered carbon^{14} converted to quercetin the results for acetate, D-glucose, and L-phenyl-lactic acid were most constant over this range but L-phenylalanine had a maximum percentage conversion or an optimal dose rate. An explanation of the results in metabolic terms has been attempted.

It is recommended that the "percentage of C^{14} converted" be used to express results in plant biosynthetic work.

Biosynthesis of phenylalanine and tyrosine in young wheat and buckwheat plan.

O. L. Gamborg & A. C. Neish, *Can. J. Biochem. Physiol.* **37**, 1277 (1959). Several C^{14}-labelled compounds were fed to shoots excised from young plants of *Tritcum vulgare* Vill. or *Fagopyrum tataricum* (L.) Gaertn. The free and bound phenylalanine and tyrosine were isolated after a metabolic period of 6 hours and their C^{14} content measured. Shikimic acid was superior to glucose and acetate as a precursor of both phenylalanine and tyrosine. Phenyllactic acid and phenylpyruvic acid were easily converted to free phenylalanine and were incorporated into bound phenylalanine as readily as was free phenylalanine itself. Similarly, *p*-hydroxyphenyllactic acid and *p*-hydroxy-phenylpyruvic acid were converted preferentially to tyrosine. There was some conversion of phenyl compounds to *p*-hydroxy-phenyl compounds but little evidence of the reverse reaction. Radioactive carbon fed as cinnamic, dihydrocinnamic, or *p*-coumaric acids was not incorporated into either phenylalanine or tyrosine.

Lipase activity in lingcod muscle preparations.

J. D. Wood, *Can. J. Biochem. Physiol.* **37**, 937 (1959). A lipase has been isolated and partially purified from the muscle of the lingcod (*Ophiodon elongatus*). Both monoglycerides and triglycerides are hydrolyzed but methyl acetate and ethyl acetate are attacked extremely slowly, if at all. The lipase is inhibited by *p*-choloromercuribenzoate but not by ferricyanide, cyanide, or azide. The pH of optimum activity is 8·2. The enzyme is unstable in aqueous solution, but can be stored at — 20°C for several weeks without loss of activity.

The combination of copper with amino acids, peptides, and proteins.

J. F. Scaife, *Can. J. Biochem. Physiol.* **37**, 1033 (1959). By equilibrating solutions containing amino acids, peptides, or proteins with the sparingly soluble copper salt malachite ($CuCO_3$. $Cu(OH)_2$); it has been possible to determine the amount of copper in the solutions complexed to these compounds, and the amount of free copper in the solutions in equilibrium with the complex. The dissociation constant for the copper-glycine complex has been estimated from the data obtained using this system. The nature of the complexes has been deduced both from chemical determinations of the bound copper, and from manometric measurements of the extent of complexing, made in bicarbonate buffers. The simple amino acids have been shown to form complexes of the type CuR_2, which are appreciably dissociated in dilute solutions. The degree of complexing is influenced by the nature of the group R. Histidine, tryptophane, and other compounds containing more than one donor nitrogen atom are able to form more than one type of complex with copper. The participation of other nitrogen atoms in complexing is related to their basicities. Glycyl-glycine is able to bind approximately twice as much copper as glycine, but the other glycine peptides from triglycine to pentaglycine show a reduced and progressively decreasing ability to bind copper.

The binding of copper to proteins differs from that of the amino acids, in that the amount of copper bound is independent of the concentration of protein present, for any given concentration of free copper. The several atoms of copper bound to each protein molecule were not all bound with the same affinity.

The catalysis of ascorbic acid oxidation by copper and its complexes with amino acids, peptides, and proteins.

J. F. Scaife, *Can. J. Biochem. Physiol.* **37**, 1049 (1959). The oxidation of solutions of ascorbic acid catalyzed by copper and the copper complexes of amino acids, peptides, and proteins has been investigated by manometric and chemical means. The copper-containing solutions were prepared by equilibration with the sparingly soluble salt malachite. The catalytic activity of the complexed copper was found to be dependent upon the type of complexing molecule. For amino acids the more firmly bound copper atoms tended to be the best catalysts. The catalytic activity of copper complexed to bovine plasma albumen and pepsin was lower than that of copper complexed with amino acids or peptides. The more firmly bound copper atoms on pepsin showed a higher catalytic activity than the mean value for all the complexed copper atoms. Sodium chloride markedly reduced the catalytic activity of both free copper and complexed copper. In the absence of sodium chloride free copper was 10 times as effective a catalyst as copper complexed to glycine. In the presence of 0·178 *M* sodium chloride the complexed copper was twice as effective as free copper.

The sorption of gaseous hydrogen chloride by dry lyophilised β-lactoglobulin.

W. A. Hnojewyj and L. H. Reyerson, *J. Phys. Chem.* **64**, 1199 (1960). The sorption of gaseous HCl by dry lyophilised β-lactoglobulin was studied and a complete adsorption isotherm was obtained at 27°C. Amounts of HCl adsorbed were determined upto 5·484 m.moles per gram of protein at an equilibrium pressure of 325·3 mm. The first equilibrium point (1·9 m.moles/gm) was reached in about three hours, the equilibrium HCl pressure being 4·515 mm. After this first rather rapid adsorption each successive point required from two to three days for equilibrium to be reached. The HCl was then desorbed very rapidly by reducing the pressure and the amounts of HCl retained at 10^{-6} mm pressure were determined at 27, 40 and 60°C. When equilibrium was finally reached, 1·540 m.moles/gm of HCl remained sorbed on the protein at 27°C. This value decreased to 1·463 and 1·1763 m.moles/gm

respectively when the temperature was raised to 40° and 60°C. The three desorptions took about 10 days for completion. The adsorbed HCl molecules had only a minor effect on the adsorption of water. The number of HCl molecules remaining adsorbed at 27°C just equalled the number of active amino groups in the side chains of this protein. The ratio of the number of HCl molecules bound at zero pressure to the number of active amino groups in the side chains of insulin and β-lactoglobulin were also determined. In the case of insulin, the ratio was greater than 1·5 at 0° but at 27°C, the ratio for both proteins was nearly unity and it fell slightly thereafter, reaching about 0·75 at 60°C, the highest temperature studied. These results were taken as a reasonable proof that the adsorbed HCl molecules were most tightly bound by the amino groups on the side chains of the protein. This binding is assumed to be of a chemical nature. The backbone peptide links did not seem to absorb many HCl molecules; if at all they did, the bonding appeared to be very weak. Unlike nylon, the protein with the maximum amount of HCl adsorbed was not hydrolysed at the peptide links when placed in water.

K. T. J.

GENERAL

Analysis of endrin residues in flour by infra-red spectroscopy.

Angela N. Bates, *J. Sci. Food Agric.* **11**, 750 (1960). A method is described for the quantitative estimation of endrin residues in flour by differential infra-red spectroscopy. The method is suitable for residues of 20-400 p.p.m. and has been applied to the analysis of flour samples which had been in contact with an endrin lacquer in a flour mill.

Insect control by gamma-irradiation: An appraisal of the potentialities and problems involved.

P. B. Cornwell and J. O. Bull, *J. Sci. Food Agric.* **11**, 754 (1960). Experimental sources of cobalt-60, made radioactive by neutron irradiation, have been used during recent years for investigating the radiation susceptibility of insect pests. Lethal and sterilising effects have been examined on species, strains and developmental stages to evaluate the dose levels required for commercial treatment. The technical feasibility of using radiation as an insecticide is now established. Results of research and problems of application are examined in relation to the two potential

methods of controlling insect pests by irradiation: indirect control by the release of sterilised adults and the direct treatment of infested products. Application of the first method, exemplified by work on screw-worm fly, is limited to very few species by a number of biological requirements. Direct treatment could have wider application for the control of all species infesting stored products. The most favourable products for radiation disinfestation are those handled in bulk at particular centres, and for this reason radiation treatment of grain has been examined in detail. The fundamental and applied problems of using γ-radiation for this purpose are discussed and the relative merits of fumigation and radiation sterilisation are compared. It is concluded that among factors which may influence the success of radiation disinfestation, bulk handling and the incorporation of treatment into established handling procedures must receive primary consideration. γ-irradiation may eventually take its place as a useful means of insect control, but it is unlikely to displace conventional methods.

NOTES AND NEWS

What happens as we grow old?

Although much remains to be learned about how metabolism in tissues and cells is affected by age, it is quite apparent that changes do occur, says Dr. Nathan W. Shock of Baltimore City Hospitals. In particular, changes take place in the highly specialized cells, which develop a reduced capacity to divide and form similar new cells.

Collagen taken from old animals is less readily solubilized than that from young animals, and it is more rigid. Skin and blood vessels become less elastic with age. As animals grow older, many of their tissues and cells accumulate calcium, cholesterol, and insoluble pigments, which indicate that their cellular metabolism has changed.

Chemical and Engineering News: January 18, 1960.

Paper chromatography broadens

Paper chromatography and ion exchange have joined hands in a new development from Rohm & Haas, Philadelphia, Pa. The development: paper loaded with ion exchange resin. R & H has it with all four types of resins—strongly acidic, strongly basic, weakly acidic, and weakly basic.

This is a union which many have tried but which no one has previously succeeded in achieving, the company says. Rohm & Haas does it by mixing finely divided resin (20 to 30 microns) with pure alpha cellulose pulp and then making paper by standard commercial techniques—and it took eight years to learn how. The final product has nothing reactive in it other than the cellulose and the resin, according to sales development engineer Robert W. Percival. But how the paper gets its wet strength, he does not say.

The final sheet contains about 50% resin and 50% cellulose. According to results from trial runs, the sheets are uniform within themselves and from run to run. Hence, the papers will be useful in precise analytical and research work Mr. Percival predicts.

Potential uses: The new resin loaded papers combine the partitioning effect of cellulose with the ion exchanging properties of resins. Thus, their uses suggest themselves—in analytical work, for anything now done by conventional ascending, descending, or circular paper chromatography, but with the added effect of ion exchange; in research, to separate problem mixtures of closely related products that either take a long time to separate or have not yet been resolved. One biochemist, for example, has used the paper to fractionate haemoglobin fractions and has discovered new components in them.

Most of R & H's development efforts have centered on research and analysis. But the new papers may have industrial uses as well:

Removing traces of exchangeable impurities during filtration.

Concentrating traces of valuable components such as noble metals.

Purifying viscous solutions such as edible oils by removing small amounts of objectionable acids or bases.

The company points out, however, that these ideas are just that—it has not had the development time to try them.

Chemical and Engineering News: January 25, 1960.

Gas chromatograph hits high °C

A new instrument that nearly doubles the scope of present gas chromatographic analysis—that's how F & M Scientific, Wilmington, Del., bills its 500°C linear-programmed temperature gas chromatograph. According to F & M, the Model 500 can:

Resolve complex organic mixtures of low-, medium-, and high-boiling materials—components with from one to 75 and possibly even 100 carbon atoms—all in one run.

Handle complex mixtures of inorganic and organic gases; organics in water; and high-boiling materials such as glycerides found in butter, margarine, and other natural products.

In addition, the instrument offers nine linear heating rates to speed up or slow down analysis time and thus give the required degree of component separation.

Chemical and Engineering News: February 1, 1960.

New process makes dialdehyde strach

The U.S. Department of Agriculture has worked out a two-stage process for making dialdehyde starch on a pilot scale. The process, which can run continuously, oxidizes starch with periodate, makes a product with over 90% dialdehyde starch content. Cost of making the product commercially by the new process: from 15·6 to 20·4 per lb., depending on plant size, says USDA.

The new process still uses the basic periodate starch oxidation reaction developed several years ago at USDA's Northern Regional Research Laboratory, Peoria, Ill. But the earlier technique used "in-cell" oxidation-oxidation and electrolytic regeneration of periodic acid in an electrolytic cell. The Peoria lab's V. F. Pfeifer and coworkers dub the two-stage scheme an "out-cell" process (I & *EC*, March 1960, page 201). Actual starch oxidation takes place in a separate vessel.

Acid making starts process: First step in dialdehyde starch making is to prepare periodic acid. USDA makes iodic acid (HIO_3) from Chilean crude iodine, then converts this to periodic acid (HIO_4) electrolytically. The cell is equipped with six lead anodes, 14 cathodes.

Oxidant is next pumped to an oxidation tank. Total equivalent iodic acid concentration is about 7%, and iodic to periodic conversion is about 85%. The starch is added and oxidation goes at pH 1·0 to 1·5 at 100°F for about three hours.

At the end of the reaction, about two thirds of by-product iodic acid is recovered by settling and decanting, and recycled to the electrolytic cell to regenerate periodic acid.

The oxidized starch slurry is centrifuged or filtered, and the effluent also pumped to the cell for periodic acid regeneration. To round out the cycle, the dialdehyde starch is washed and dried.

USDA says the operation can be put on a continuous basis. It does this by putting the oxidizing solution through the cells with series flow to make periodic acid. The solution then mixes with starch or a starch slurry, passes through a series of tanks for three to four hours retention. A continuous centrifuge concentrates the reaction slurry, and product is finally separated in a bank of basket centrifuges.

Out-Cell vs. In-Cell:—Advantages claimed for the two-stage process compared with in-cell oxidation include:

(a) Improved control over both process and product.

(b) Lower production costs; cell construction is simpler.

(c) Less lead contamination (from anodes).

(d) Higher current efficiency.

Chemical and Engineering News: March 7, 1960.

No-waste chemical pulping?

A team of chemists at University of California has come up with an idea that could spark renewed chemical pulping interest in that state. The scheme: a virtually no-waste, nitric acid-ammonium hydroxide process to make pulp from wood chips, sawdust, and other residues from the state's huge logging operations. And, in the bargain, it converts the effluent into fertilizer for ready nearby markets.

The new concept—still in its infancy—is an offshoot of the old nitric acid pulping process, Dr. David L. Brink told the Technical Association of the Pulp and Paper Industry in New York. Major switch: ammonium hydroxide is used instead of sodium hydroxide. In a first-stage reaction, wood chips are treated with nitric acid, which partially solubilizes and partially dissolves some of the wood. The spent acid liquor, which contains residual nitric acid and organic acids, is drawn off and held, while the treated wood chips are extracted with ammonium hydroxide. The second-stage ammonium hydroxide treatment removes the wood material that was solubilized in the acid stage—namely, part of the lignin and some of the carbohydrates.

The two solutions—acidic from the first stage, basic from the second—are combined. The excess ammonium hydroxide neutralizes the acid extract, and the solution is distilled to remove any excess ammonium hydroxide. End product: a virtually neutral solution of ammonium salts of organic acids and some residual nitric acid that can be concentrated, or used as is, for fertilizer.

Chemical and Engineering News: March 14, 1960.

Metal-8-quinolinolates for fungicides

A comparative study of ten laboratory-prepared metal—8—quinolinolate compounds to determine their effectiveness as protective treatments for fabrics exposed to weather has been conducted at the USDA's Southern Utilization Research and Development Division in New Orleans, La.

The studies were a part of the cooperative programme of research by the Canvas Products Association International and the Southern Division.

Copper-8-quinolinolate as a fungicide for canvas awnings and other fabrics exposed to outdoor weathering is said to meet most of the requirements for such treatments, except that it imparts a yellowish-green or light olive drab colour to the fabric, which is objectionable in some light stripped awnings and other decorative outdoor fabrics.

Other metals selected for this study were aluminium, bismuth, cadmium, chromium, cobalt, copper, iron, manganese, nickel and zinc. The 8-quinolinolate salts of these metals were prepared, classified as to colour, exposed, and evaluated for this fungicidal effectiveness as well as for their effect on the rate of fabric degradation.

Colours of the treated canvas ranged from dark gray imparted by the iron salt to cream, produced by the manganese salt, and the lightest, a very light yellow produced by the nickel salt. Commercial and laboratory-prepared copper 8, and zinc and aluminum 8 showed no visible mildew growth at the end of the weathering period. The fabric treated with commercial copper 8 showed the highest level of strength retention (69-70%).

American Dyestuff Reporter: September 19, 1960.

U. K. takes about 50,000 raw Indian goatskins in August

Robert Mills & Company (London) Ltd. advise that during the month of August, 1960, approximately 812,000 pieces Indian goatskin were exported from Calcutta (compared with about 900,000 for the same period last year).

Drysalted: About 595,000. To the U. K. about 44,000; Continent about 516,000; U.S.A. about 35,000.

Wetsalted: About 217,000. To the U.K. about 11,000; Continent about 171,000; U.S.A. about 35,000.

Kidskins: Nil.

Of the overall total about 565,000 pieces were for Eastern European countries.

Leather Trades Review: October 5, 1960.

Use of compressed air for tannery effluent

The recent growth of Athens brought the tanneries of Cavada Brothers Limited within an inhabited area, and the firm was requested by the city hygiene department to purify the effluents from the factory before they were discharged into the municipal sewage system.

Tannery waters, as every tanner knows, contain, in addition to germs and dirt, large quantities of albumen (from the animals' blood), gelatin (from the skin), proteins and remains from tannate substances such as $Ca(OH)_2$, NaCl, Na_2S and Na_2SO_4. They also smell very disagreeable.

At first, Cavada Brothers tried to purify their trade wastes, but without success. Next, chemical purification assisted by mechanical agitation was attempted, but was abandoned because oxidation was not complete, corrosion resulted in heavy maintenance costs, and enormous power was needed to drive the agitation machinery.

The problem was brought to the attention of Atlas Copco (Greece) Limited, who suggested the use of compressed air as a purifying medium.

With the agreement of the chief chemical engineer of the tannery, Atlas Copco (Greece) helped him to design two large concrete tanks each of 5,250 cu. ft. capacity. Compressed air mains were connected to the bottom conical orifices of these tanks.

The system of cleansing is as follows: the tannery effluent is pumped into the tanks and mixed with chloride of lime and chromate salts. The mixture is agitated for six hours by compressed air provided by an Atlas Copco KE6RAS compressor. The mixture is left to settle until the cone of the tanks is filled with precipitate and the water above is odourless and pure.

Precipitation period is between one and two hours. The compressed air not only agitates the water/chemical mixture, but also provides oxygen for complete oxidation. The method has been approved by the Athens hygiene department.

After settlement, the purified effluent is piped off to the sewage main and the precipitate stored in an underground tank and removed periodically for dumping in an unpopulated part of the countryside outside the city.

The air is conveyed from the air receiver to the tanks by a ¾" pipe ending in a ¾" copper hook (one inside each tank) perforated with eight 4 mm. holes.

Leather Trades Review: October 19, 1960.

Exports of hide and skin to U. K.

Bulk of hide and skin shipments from Madras during September came to U. K. when out of a total of over 4½ thousand bales the U.K. received 3,012. These consisted of: 2,202 cow hides, 94 calves, 65 buffalo hides, 99 buffalo calves, 218 goat, and 334 sheep. Over 800 bales went to the Continent. They were: 50 cow hides, 65 calves, 10 buffalo calves, 486 goat and 227 sheep. Other main destinations were the U.S., Japan and Eastern European countries.

Leather Trades Review: October 19, 1960.

Chrome upper leather shortage in Ceylon

There is still a considerable shortage of chrome upper leather in Ceylon despite the fact that local tanners doubled output in 1959.

Buffalo hide tanning for heavy sole leather was also intensified and increased eight-fold with, it is claimed, the attainment of British standards.

With higher leather production, Ceylon is relying more on local shoe manufacturers to provide shoes to meet rising demand and recently a 35 per cent import duty increase was imposed on imported shoes.

Leather Trades Review: October 19, 1960.

Hide and skin shipments from Madras, September 1960

Destination	Td. Cow Hides	Td. Cow Calf	Td. Buff Hides	Td. Buff Calf	Td. Goat	Td. Sheep
U.K. ..	2,163	94	65	99	218	334
U.S.A. ..	—	—	80	53	244	—
Continent ..	59	63	—	10	491	218
Japan ..	—	—	—	—	2	246

Shoe and Leather Record: October 7, 1960.

Shipments of hides and skins during December, 1960.

Shipments during December were as follows: 2,505 bales tanned cow hides, 135 bales calfskins, 33 bales buff hides, 195 bales calfskins, 429 bales goatskins, 492 bales sheepskins to the U.K., 10 bales cow hides, 103 bales calfskins, 83 bales buff calfskins, 740 bales goatskins, 430 bales sheepskins to the Continent, 65 bales buff hides, 45 bales calfskins, 178 bales goatskins, 5 bales sheepskins to the U.S.A., 60 bales sheepskins to Japan, 5 bales cow hides to Aden, 7 bales sheepskins to Australia, 10 bales goatskins, 11 bales sheepskins to New Zealand. Total 5541 bales.

The Mail: January 8, 1961.

Maintenance of cattle

Inaugurating the 20th annual meeting of the Tanjore S.P.C.A. and the fourth Tanjore district cattle show, Mr. M. Bhaktavatsalam, Home and Agriculture Minister, Madras, said that greater attention should be paid to the maintenance of cattle. To satisfy the demand for milk, the Government had set up cattle farms to upgrade the quality of cattle. He referred to the centre at Orathanad in Tanjore district to upgrade the stock of the Umblacheri breed.

The Mail: January 21, 1961.

C.L.R.I. NEWS

Dr. Y. Nayudamma, Director, has been nominated as a member on the Board of Studies in Technology of the Madras University for a period of 3 years from January 1, 1961 to December 31, 1963.

Dr. D. Ramaswamy, Senior Scientific Officer, resumed charge at the Institute on January 7, 1961 after higher training for an year in the field of "Radioactive tracer techniques and metal complex chemistry" in the U. K. under the Colombo Plan.

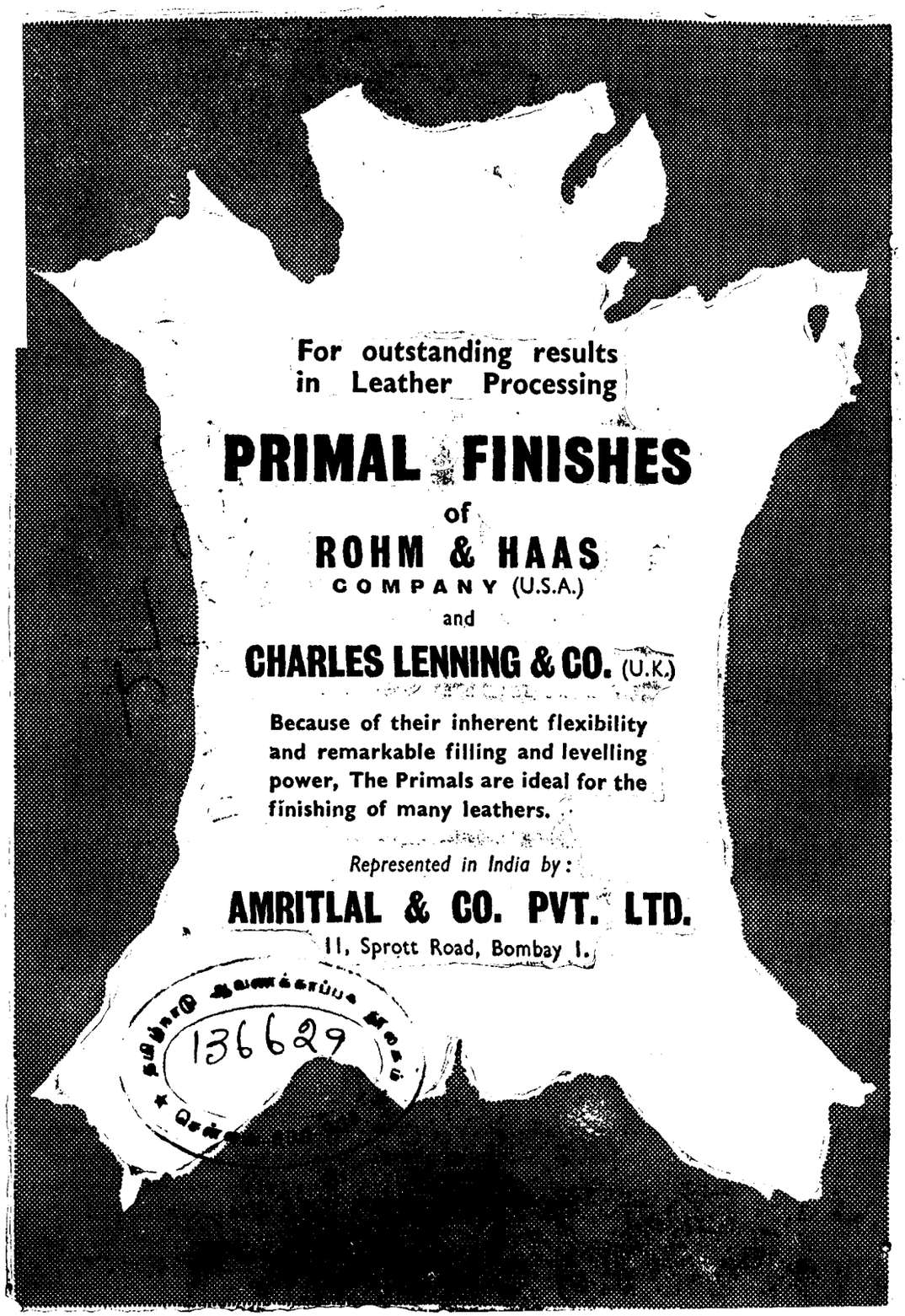
Dr. Y. Nayudamma, Director, attended the Governing Body meeting of the Madras Productivity Council on January 24, 1961.

A meeting of the Board of Visitors for the Institute of Leather Technology, Madras, was held on January 25, 1961, with Dr. Nayudamma as the Chairman.

Mr. M. A. Ghani, Senior Scientific Officer, attended the Technology Sub-Committee meeting of the Indian Central Arecanut Committee at Madras on January 19, 1961 as a representative of the Director.

The Public Accounts Committee of the Lok Sabha who visited the Institute in January 1961

Editor Y. Nayudamma. Published by N. Subramaniam, on behalf of the Central Leather Research Institute, Adyar, Madras-20. Printed by G. Srinivasachari, B.A., at The G. S. Press, 21, Narasingapuram Street, Mount Road, Madras.


For outstanding results
in Leather Processing
PRIMAL FINISHES
of
ROHM & HAAS
COMPANY (U.S.A.)
and
CHARLES LENNING & CO. (U.K.)
Because of their inherent flexibility
and remarkable filling and levelling
power, The Primals are ideal for the
finishing of many leathers.
Represented in India by:
AMRITLAL & CO. PVT. LTD.
11, Sprott Road, Bombay 1.

"Registered No. M. 7050."

CLRI Publications

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

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

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

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

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

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

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

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

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

SYMPOSIUM ON "UTILISATION OF BY-PRODUCTS OF LEATHER INDUSTRY" (1960): Proceedings and Papers. Re. 1/- per copy. Postage extra.

For further information please contact

Director

CENTRAL LEATHER RESEARCH INSTITUTE

Adyar, Madras-20, India