

A PROGRESS REPORT ON THE ENZYME DEPILATION OF CATTLE HIDES*

1277

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ABSTRACT

In a survey of some 40 enzyme preparations for hair-loosening activity, several possessing rather marked activity were found. The relation of depilatory action to hydrolytic activity in these active preparations is shown. The conditions for use of one of the most active preparations are given, and some special equipment devised for studying enzyme activity on hides and skins is described.

Results of tests in which whole hides were treated with enzymes and unhaired on conventional unhairing machines are presented, and the problems encountered are discussed.



INTRODUCTION

The need of the tanning industry for a rapid, economical method of removing the epidermal system from animal hides in preparation for tanning and at the same time alleviating the problem of the disposal of used lime-sulfide liquors from the present unhairing systems is becoming increasingly acute. In a continuation of our previous studies (1) a survey was made of some commercially available enzymes for their depilatory action on steer-hide. The relation of depilation to proteolytic and amylolytic activity is reported elsewhere (2, 3).

MATERIALS AND METHODS

Hides.—The hides used were all from commercial sources. The data given in Tables I and II were obtained from Delph** hides which were obtained from a local tanner. These hides were demanured, fleshed and brine-cured commercially, then stored at the laboratory in moisture-proof bags at

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4°-6°F. until needed. Six-inch strips were cut parallel to each side of the backbone in the bend area and two- to three-inch squares were used. They were soaked overnight in a disinfectant solution just prior to treatment. The disinfectant used in the soak water was a 0.03% solution of BSM-11, a commercial disinfectant, which contains 10% phenylmercuric acetate and 50% potassium 2,4,6-trichlorophenol in undisclosed solvents.

For the data in Table III pieces cut from a commercial green-salted steer-hide and calfskin were soaked overnight in a disinfectant solution before use. The goatskin was soaked in a tannery and kept frozen until needed.

The hide pieces used to obtain the data in Tables IV, V, and VI were taken from green-salted hides which had been hand-fleshed, soaked overnight in a disinfectant solution, and kept frozen until needed.

In the full-hide tests green-salted hides were washed in a drum for 10 to 20 minutes, fleshed on a machine, resalted, and stored in moisture-proof bags at about 4°-6°F. until needed.

Estimation of hair loosening.—Hair looseness was estimated by feel and rated from 0 to 5+. For more accurate work the device shown in Fig. 1 was used. The blade, under a constant weight, was pulled repeatedly

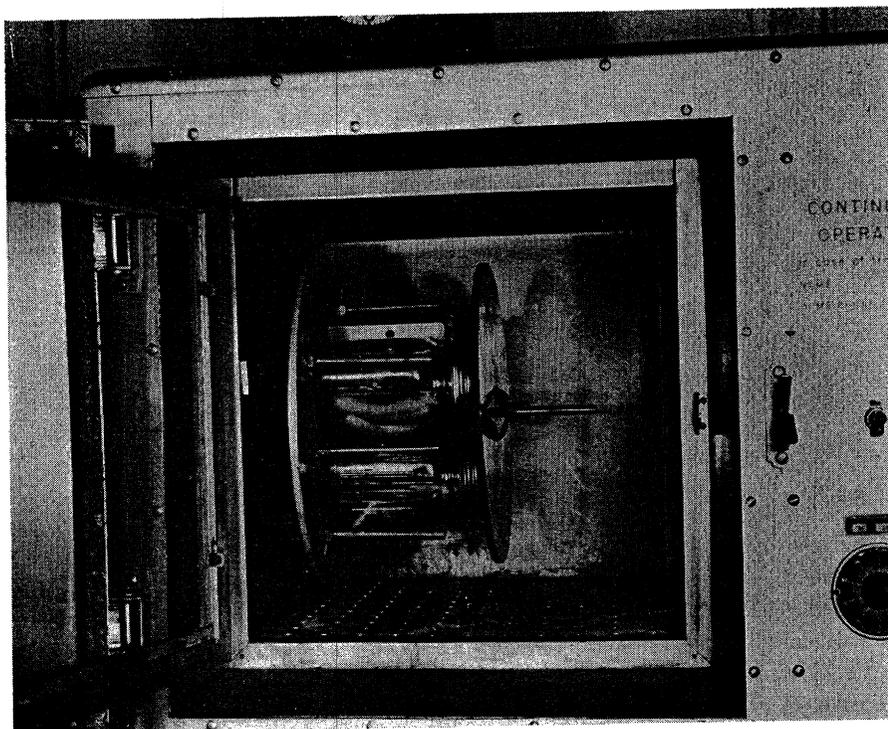


U.S.D.A. Photo by M. C. Audsley

FIGURE 1.—Device for testing the degree of hair loosening of enzyme-treated hides. The instrument measures 9" in length, has a blade 1" wide, and exerts 470 grams weight at knife edge.

over the treated hide until no further hair was removed. The number of pulls and the estimated percent of hair removed were recorded.

Special equipment.—Mild agitation has been found to increase the rate of hair loosening. An oven was equipped with a device to rotate small samples of hide in various-sized jars (Fig. 2). An interval timer was used to



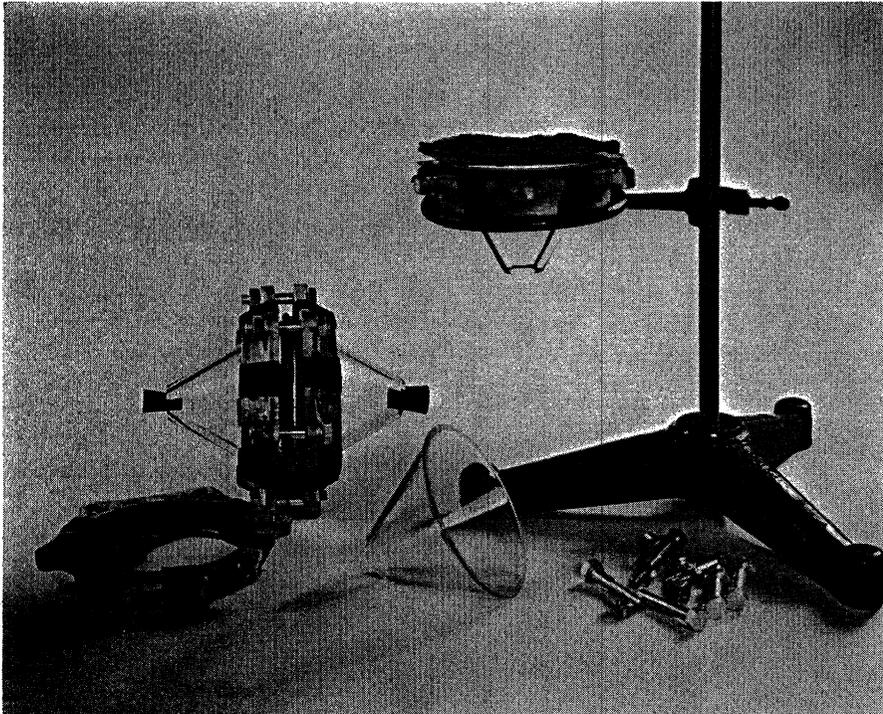
U.S.D.A. Photo by M. C. Audsley

FIGURE 2.—Oven equipped with device for rotating hide samples during treatment.

regulate the time the rotor operated. With this device it was possible to control the amount of agitation and the temperature.

In order to determine whether the high proportion of edges on small pieces of hide would influence the rate of unhairing, a device (Fig. 3) was assembled by which the edges were kept out of contact with the unhairing solution. This device consisted of two 4" funnels with the large-diameter edges ground plane and the stems cut off at a point on the walls so that openings large enough to introduce the solutions would be present. In use the hair was clipped from the edge of 4½"-diameter pieces of hide, and the hide was placed between the funnels, which were held together with aluminum clamps made for assembling glass piping.

ENZYME DEPILATION OF CATTLE HIDES



U.S.D.A. Photo by M. C. Audsley

FIGURE 3.—Device for isolating the hair and flesh surfaces of a hide or skin for independent treatment.

Enzymes used.—From some 40-odd enzymes which were surveyed for depilatory activity (2, 3), the following were included in these tests: HT Concentrate and HT Proteolytic (bacterial enzymes from the Takamine Laboratories), L-56D (a bacterial protease from the Pabst Laboratories), Protease 15 Concentrate (a bacterial enzyme from Rohm & Haas Company), 4511-3 (a bacterial enzyme from the Wallerstein Company), Papain and Bromelin (plant enzymes), and trypsin and pancreatin from the pancreas. These enzymes are described more fully in Table I.

Enzyme assays.—Protease (PV) activity was measured using an adaptation of the Gross-Fuld method (6). The method is based on the principle that the degree of digestion of a casein solution by a proteolytic enzyme, conducted under standard conditions, is proportional to the activity of the enzyme. The digested casein solution, upon acidification, produces a turbidity which is inversely proportional to the degree of digestion. This turbidity is measured with ease and reliability in a photoelectric colorimeter. One PV unit is defined as the quantity of enzyme which digests 1 mg. of casein to

TABLE I
RELATION OF HAIR LOOSENING TO OTHER ACTIVITIES
OF SOME SELECTED ENZYME PREPARATIONS

Enzyme	PV(a) Units/g.	Formol Ti- tration(b) meq.-N/g.	DV(c) Units/g.	Concentration Used g./100 ml.	Hair Looseness P(d) R(e)
HT Concentrate(f)	190,000	46.0	95,000	0.1	7-84
HT Proteolytic(f)	331,000	41.8	9,200	0.06	7-83
L-56D(g) Protease 15	100,000	35.5	4,500	0.19	7-84
Concentrate(h)	162,000	26.7	2,200	0.12	8-77
4511-3(i)	46,300	19.1	17,000	0.41	9-76
Papain	24,800	10.0	<300	0.77	3-97
Bromelin	46,200	46.3	<400	0.41	4-91
Pancreatin 3 X USP	66,800	36.6	16,300	0.28	10-25
Trypsin 4 X USP Pancreatin	66,500	38.6	3,200	0.29	10-0

- (a) Action on casein.
 (b) Action on gelatin.
 (c) Starch-dextrinizing power.
 (d) Number of pulls with scraper. See Fig. 1.
 (e) Estimation of percent of hair removed.
 (f) Bacterial enzymes from the Takamine Laboratories.
 (g) Bacterial enzyme from Pabst Laboratories.
 (h) Bacterial enzyme from Rohm & Haas Company.
 (i) Bacterial enzyme from Wallerstein Company.

the "standard turbidity end point" in one hour at 37°C. and pH 7.0. The end point corresponds to 78% transmittance through 7/8" absorption test tubes in the Evelyn photometer at 420 m μ .

The dextrinizing value (DV) of the enzyme preparations was measured photometrically using the Tappi suggested method T 643 sm-54(5). One DV unit represents the enzyme activity equivalent to the dextrinization of 20 mg. of Lintner starch in 30 minutes at 30°C. and pH 6.6.

The formol titration values were obtained by allowing the enzymes to react with gelatin solutions under controlled conditions, adding formaldehyde, and titrating with standard sodium hydroxide.

LABORATORY RESULTS

The 40-odd enzyme preparations which were surveyed (2, 3) showed a wide divergence of hair-loosening action. Results with some of the more effective preparations are reported here. Pieces of hide 3" square prepared as described under Materials and Methods were completely submerged in enzyme solutions whose concentrations were chosen to give 19,000 PV units per 100 ml. of solution. The temperature was maintained at 40°C., and incubation was carried out for 17 hours. With the exception of papain the pH values were between 6.2 and 6.5. In order to activate the papain, 0.1M Na₂S₂O₅ was

used, and the pH of this solution was 5.4. The sulfite in the papain solution also served as a disinfectant. All the other solutions contained 0.015% phenyl mercuric acetate as a disinfectant.

Table I shows the relative unhairing activity of nine enzyme preparations and its relation to some other activities of the preparations. The hair-looseness values are averages of 12 determinations. The concentration of the enzymes was adjusted to give the same PV activity as a 0.1% HT Concentrate which was used as a reference. Thus, if the PV activity were also a measure of hair-loosening activities, then the results obtained should have been the same for all cases. This is obviously not the case. Also the formol titration values, which reflect a type of proteolytic activity on gelatin, are not proportional to the casein digestion values nor do they show any relation to the degree of hair loosening. It is apparent, then, that neither of these assays measures the hair-loosening activity of the enzyme preparations.

The comparatively high hair-loosening value for papain may be partly explained by the large amount used. It is quite possible that the PV activity as determined by the Gross-Fuld procedure does not represent the true activity for papain. It was observed that the natural acidity of the papain lowered the pH of the solution to the point where the casein was precipitated, probably resulting in lowered readings.

There is no apparent correlation between hair loosening and the starch-dextrinizing values (DV) of the enzymes.

On the basis of the hair-loosening results given in Table I the amount of each enzyme required to give equivalent hair loosening was estimated. The values are shown in Table II. Experimental conditions were the same as those given for Table I. For the most part fairly good agreement was obtained. It is believed that these results could serve as a guide for the use of these enzyme preparations in large-scale tests.

Enzyme penetration.—The question has often been raised as to the direction of penetration of the enzyme into the hide and whether much of the activity might not be through the edges when small pieces of hide are used. The device shown in Fig. 3 was used to help resolve these questions. It was found that hide pieces unhaird equally well whether the edges were exposed to the solution or not.

Table III shows the results of penetration studies using 0.1% and 1.0% solutions of HT Concentrate in contact with the hair or flesh side of the hide or skin and rotated in the oven at 40°C. for 17 hours. Under these conditions both concentrations of enzyme loosened the hair from the hide and skins when applied to the hair side. There were differences when the enzyme was applied to the flesh side, and they probably reflect the effect of skin thickness. Neither concentration of enzyme loosened the hair from the steerhide when applied to the flesh side. A 0.1% solution did not penetrate

TABLE II
CONCENTRATION REQUIREMENTS FOR EQUIVALENT HAIR
LOOSENING BY SOME SELECTED ENZYMES

Enzyme	Amount Enzyme Used g./100 ml.	Hair Looseness			Average P R
		Experiment No.			
		1 P* R†	2 P R	3 P R	
Papain	1.0	4-98	2-98	3-98	3-98
Bromelin	0.7	3-97	3-97	2-99	3-98
HT Proteolytic	0.15	3-95	3-97	3-99	3-97
HT Concentrate	0.25	3-98	3-99	3-97	3-98
L-56 D	0.40	5-95	3-97	2-98	3-97
Protease 15 Concentrate	0.70	3-98	2-98	4-96	3-97
4511-3	1.50	5-96	3-98	3-98	4-97
Pancreatin 3X USP	2.00	3-99	3-94	3-96	3-96
Trypsin 4X USP	2.00	3-97	3-98	3-95	3-97
Pancreatin					

*Number of pulls with scraper. See Fig. 1.
†Estimation of percent of hair removed.

TABLE III
HAIR LOOSENING BY PENETRATION OF ENZYME FROM
HAIR VERSUS FLESH SIDE OF HIDES AND SKINS

Kind of Skin	Enzyme*		Hair Loosening
	In Contact With	Concentration % Solution	
Steer	Hair	0.1	moderate
		1.0	strong
	Flesh	0.1	none
		1.0	none
Calf	Hair	0.1	moderate
		1.0	strong
	Flesh	0.1	none
		1.0	moderate
Goat	Hair	0.1	moderate
		1.0	strong
	Flesh	0.1	moderate
		1.0	strong

*HT Concentrate

calfskin from the flesh side, but a 1.0% solution did. Both concentrations loosened the hair on goatskin from the flesh side.

Since the enzyme is not required to be in contact with the flesh side of the hide to effect hair loosening, experiments were run in which the hair side was simply dipped in or sprayed with the enzyme solution. Pieces of salt-cured hide which had been soaked overnight in a disinfectant solution were run through a clothes wringer to remove excess water. Weights taken before and after dipping the hair side showed the pickup to be about 8% to 10% of the hide weight. The amount of pickup depends on factors such as the residual moisture and the amount of hair. Some other hides tested have held about 15% of solution.

The treated hide pieces were stored in a humid atmosphere and at controlled temperatures. Because of the difficulty of measuring accurately the degree of hair loosening and the variation of individual hides it is not possible to make a hard and fast statement, but usually the hair on salt-cured steer-hide dipped in 1% HT Concentrate and held at 45°C. was loose enough to be easily scraped off in about 3½ to 4 hours.

Conditions affecting hair loosening by HT Concentrate.—One of the enzyme preparations, HT Concentrate, was studied in some detail to learn the best conditions for its use.

TABLE IV
EFFECT OF CONCENTRATION ON DEGREE OF HAIR LOOSENING
BY HT CONCENTRATE

Concentration*	Agitated 40°C., 16 hr.	Not Agitated 45°C. Hours					Not Agitated 25°C., 16 hr.
		1	2	3	4	5½	
		Hair Looseness†					
0.075	4+						
0.10	5+						
0.15	5+						
0.25		0	+	2+	3+	4+	4+
0.50		0	+	2+	3+	4+	5+
1.00		0	3+	4+	4-5+	5+	5+
		0	4+	5+			

*Percent in solution.

†Rated 0 to 5+ where 5+ = hair completely loose.

Concentration.—The results reported in Table IV show that the rate of hair loosening increases as the concentration is increased up to a 1.0% solution. Raising the concentration above 1.0% does not speed up the rate of hair loosening. Experience has shown that the hair on green-salted steer-

hides can be loosened sufficiently for easy unhairing by the action of 0.1% HT Concentrate in 16 to 24 hours at 40°C. with mild, intermittent agitation or in 3 hours by a 1.0% enzyme concentration even without agitation.

pH—The effect of hydrogen ion concentration between pH 3.5 and 9.0, on the unhairing activity of HT Concentrate was studied. Sodium acetate-acetic acid and phosphate buffers were used. Neither buffer system appeared to affect the enzyme activity. Table V shows that the unhairing activity was approximately equal between pH 5.5 and 7.0. Beyond these

TABLE V
EFFECT OF pH ON HAIR-LOOSENING ACTIVITY BY 0.08% HT CONCENTRATE IN ACETIC ACID-SODIUM ACETATE OR PHOSPHATE BUFFERS

pH of Buffer	pH at Finish	Hair Looseness* after 16 hours at 40°C.	Condition of Hide
3.50	3.58	+	plumped, turgid
4.07	4.29	3+	less plumped, much less turgid
4.67	4.90	3+	same as previous
5.10	5.29	4+—5+	firm, not turgid
5.75	6.01	5+	" " "
6.10	6.21	5+	soft, somewhat flaccid
7.00	6.51	5+	soft, flaccid
7.45†	7.11	5+	" "
9.0 †	7.53	3+	not flaccid

*Rated 0 to 5+ where 5+ = hair completely loose.

†Phosphate buffer.

limits it fell off rapidly. The condition of the hide was affected by the pH of the unhairing solutions. Above about pH 6.0 the hide became soft and flaccid, below this point it became increasingly swollen and firm. The swelling of collagen in acid or alkaline solutions is well known (4), and the swelling in this case no doubt reflects the acidity present. However, histological studies have shown that in the higher pH range the elastin was largely destroyed, whereas in the lower range very little attack on this component could be seen. The pH at which the unhairing is carried out might play a significant role in determining the nature of the leather which could be produced from the unhairing hide.

Temperature.—As shown in Table VI, increasing the temperature causes a marked increase in the rate of hair loosening. Temperatures much above 45°C. cause damage to the hide and hence cannot be used.

Hair loosening does take place at 6°C. but at a much reduced rate. After 8 days the hair was completely loose at each concentration shown in Table VI. An interesting phenomenon was observed at this temperature in that

TABLE VI
EFFECT OF TEMPERATURE ON THE RATE OF HAIR LOOSENING
WITH HT CONCENTRATE
(No agitation, 19 hours)

Enzyme Concentration Percent in Solution	Hair Looseness*				
	Temperature °C.				
	45	30	24	18	6
0.05	4+	3+		+	0
0.10	5+	4+	4+	2+	+?
0.25	5+	5+	4+	4+	+?

*Rated 0 to 5+ where 5+ = hair completely loose.

the entire epidermal layer, including hair follicles and sebaceous glands, was removed intact. This may indicate that at low temperatures a different type of enzyme action takes place.

TESTS WITH FULL HIDES

In view of the promising laboratory results, several tests using full hides were run, and the unhaired hides were tanned into leather of various types. It was found that bacterial growth must be adequately controlled during soaking or severe damage will result when the hide is placed in the enzyme solution and held at a temperature favorable to enzyme action. This temperature is also ideal for bacterial growth. This factor is not so critical when lime is used for unhairing because the high pH of the lime inactivates the bacteria.

Upholstery leather.—In this test six steerhides were soaked 24 hours in water containing BSM-11 disinfectant, 1 part to 1,000 parts hide, then fleshed and placed in a paddle vat in 250 gallons of 0.1% HT Concentrate enzyme based on the weight of the water. Acetic acid and sodium acetate were added to buffer the solution at about pH 5.5. Preservation was effected by using the same disinfectant and concentration as in the soak. The temperature was adjusted and maintained at 38° to 40°C. using live steam. The dilution caused by steam condensation was compensated for by adding calculated amounts of enzyme, buffer salts, and disinfectant. The paddle was run 5 minutes each hour.

This regimen was maintained for about 16 hours at which time most of the hair had come off. However, there was some fine hair still remaining, and in order to further loosen it enough additional enzyme was added to bring the total to 0.15%. The temperature was raised to 43°-45°C., and the paddle was run continuously. After four hours, at which time the hides had been in

the enzyme solution for 21 hours, they were removed and run through an unhairing machine. Unhairing at this tannery was carried out by a sulfide pulping system, and the machine was set for the swollen, turgid hides which that treatment produces. Although it was not possible to change the setting on the machine to accommodate the flaccid hides produced by the enzyme treatment, practically all the hair, including most of the fine hair, was removed.

Three of the hides were bated overnight and three placed directly in the vegetable tanning liquor with the regular stock. The upholstery leather produced was rated satisfactory by the tanner, but some tanners found the grain splits somewhat flat. The flexibles were termed excellent. No difference could be noted in the leather from the bated and unbated hides.

Side upper leather.—These tests were run at the Lowell Technological Institute in cooperation with Dr. Thomas Thorstensen. HT Concentrate was compared at 0.1% and 0.2% concentration in paddle vats using five pairs of sides which had been soaked in disinfectant solution overnight. Difficulty was experienced in maintaining the temperature of the vats, and the hair was not so loose as had been obtained in laboratory tests. There was no noticeable difference between the two treatments. The hair was removed by hand-beaming with medium to firm pressure, although it was thought not to be quite loose enough for unhairing on a commercial unhairing machine.

In another experiment stronger enzyme solution (1%) was sprinkled on the hair side of two sides which were then folded and allowed to stand overnight. The hair on these sides was probably loose enough to be removed on an unhairing machine. All the hides in these tests were unhaired by hand on a beam.

The unhaired hides were rather flaccid, and the grain appeared to be loose. They were placed in a sulfuric acid-sodium chloride pickle overnight, then taken to a tannery, repickled, and made into chrome-retan shoe upper leather. Some tanners found the finished leather satisfactory, others thought it was somewhat flat and firm. Contrary to the condition observed at the time of unhairing, there was no indication of looseness of grain. As a result of these preliminary tests it appears that some modifications will have to be introduced in the tanning process to yield leathers comparable to present commercial production.

Sole leather.—A series of unhairing tests was run with whole hides which were tanned to produce sole leather. Green-salted hides, prepared as described above, were soaked overnight in a disinfectant solution and treated in various ways with solutions of HT Concentrate enzyme.

Several variations of treatment with the enzyme were tested, and although it was possible to loosen the hair to what appeared to be a satisfactory extent,

the standard unhairing machines would not remove all the fine hair. However, in many instances this hair fell out in subsequent processing.

These unhairing hides could be processed through a regular sole leather tanyard after a brief liming (one day); however, most of these were tanned without liming by a modified experimental procedure. Some bends were comparable in physical properties and chemical analysis to conventional sole leather.

DISCUSSION

A number of enzyme preparations have been found which will loosen the hair on animal hides. There may be some qualitative differences in their action on hides, because they show marked differences in activity as measured by various assays. Whether the quality of the leather produced will be dependent upon the specific nature of the enzyme used for unhairing remains to be determined.

Among the problems which need to be solved is that of removal of fine hair. There is evidence that this fine hair may be just as loose as the long hair after enzyme treatment but that because of the soft flaccid nature of the hide, the standard unhairing machines do not remove it. There are several ways this problem might be solved. We have noticed, as have others, that in washing, paddle-bating, etc., the fine hair will come out. Perhaps this is all that is necessary. However, we are now experimenting with other ways of removing the hair. A brush in place of the unhairing blades seems to hold promise.

Another perplexing problem is the inconsistency of results. An unhairing procedure may give excellent results with one trial and poor results at another time without any apparent change in the conditions. This may be due to some undetected factor that is not being controlled or perhaps merely to differences in the raw stock. We hope to find out.

We are well aware that the hides produced by enzyme unhairing differ both chemically and physically from limed hides and that changes in processing will undoubtedly have to be made to produce high-quality leather. Much of our effort will be directed toward this end.

SUMMARY

Nine enzyme preparations, selected for depilatory activity from a recently surveyed group of 40, are compared as to their ability to act upon casein, starch, and gelatin. Their comparative hair-loosening ability is also reported. Some special equipment is described. Results showing the direction of penetration of enzymes into hides and skins are given. The effects of concentration, pH, and temperature on hair loosening by one enzyme preparation are reported.

Some larger-scale tests in which whole hides were treated with enzymes and unhaired on conventional unhairing machines are presented. Some of the problems encountered and possible solutions are discussed.

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REFERENCES

1. Cordon, T. C. *JALCA*, **50**, 270-74 (1955).
2. ———, H. W. Jones, A. L. Everett, and I. D. Clarke. *Bacteriol. Proc.*, **1957**, 26.
3. ———, ———, I. D. Clarke, and J. Naghski. *Appl. Microbiol.*, **6**, 293-97 (1958).
4. McLaughlin, G. D., and E. R. Theis. *The Chemistry of Leather Manufacture*. ACS Monograph No. 101. New York: Reinhold Publishing Corporation, 1945.
5. Tappi Suggested Method T 643 sm-54, Dextrinizing Value of Enzymes (Enzyme Activity), *Tappi*, **37**, 113A (1954).
6. Tauber, H. *The Chemistry and Technology of Enzymes* (New York: John Wiley & Sons, 1949) p. 181.

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DISCUSSION

DR. TURLEY (Rohm & Haas Company): To those who work in this field this is a fascinating process and also full of difficulties. I think if the enzyme methods of unhairing had been relatively simple and straightforward, we would have had successful methods for removing the hair by means of enzymes a long time ago and they would possibly be used widely in the industry.

Of course the enzyme methods go back to World War I and originated in Germany, and they did encompass using alkaline swelling agents prior to the enzyme. They had a limited success and more or less died out. And then this whole field was resuscitated again in 1953, by Burton, Reed, and Flint in England.

Due to the changed situation today with regard to tannery effluents the whole subject has become important again. Burton—and probably Dr. Flint, who worked with him—stressed the question of mucopolysaccharides and the use of mucolytic enzymes. Some of them gave positive results, but I believe the results were due more to the proteolytic enzymes that the

products contained, since they were commercial products. Since that time Dr. Cordon and his school are finding that the diastatic activity has no relation to the unhairing activity, and I imagine that idea will be dropped.

Further, there is the difficulty in relating unhairing action actually with the proteolytic activity of the enzyme as measured by what we used to call the Fuld-Gross method, but is now referred to here as the Gross-Fuld method. There will probably have to be a breakdown into some other types of proteolytic enzymes.

Dr. Cordon is quite frank to admit that one of the problems is the inconsistency of the results. Perhaps in discussion here somebody might be of help in that direction and shed some light on some of these problems. As far as inconsistency is concerned, I wonder whether Dr. Cordon has had in mind the effect of the autolytic enzymes present. They may be operative under some conditions and depressed under other conditions. I say that because of the interesting results that are obtained at quite low temperatures, given enough time, by using no enzymes at all. Apparently under some conditions the hair will slip very readily. It might be due to residual autolytic enzymes.

Another important point is the question of the quality of the leather. No process using enzymes is going to be successful if good quality leather cannot be made. It is rather interesting to see that the leathers made with the vegetable process were considered satisfactory but that those put through the chrome process were not considered satisfactory. I am going to ask Dr. Cordon if he has anything to say on that.

I would like to say this, just before throwing the meeting open to general discussion: There has been a lot of talk about the new enzyme unhairing processes of today. But I am not aware whether in Europe or in this country there is yet a successful process which will operate under practical conditions, day in and day out, on large quantities of hides. Those problems are still in front of these workers. I want to point that out.

A first question—the question of the autolytic enzymes; and second, the difference between the vegetable leather and chrome leather, if Dr. Cordon will say a few words on it.

DR. CORDON: I have observed that on occasion the hair becomes loose without any enzyme being present. One very striking thing happened. We had a piece of hide that was frozen in a deep-freeze chest. We sampled it periodically over a year and a half and noticed that as samples were taken they became increasingly easy to unhair. Just recently we took a piece out and thawed it and found no enzyme was needed to loosen the hair. You could brush the hair off, epidermis and all. It had been kept frozen at -10°C .

in a deep-freeze chest all this time. So something does go on. I have noticed other cases where the hair has become loose sitting in a disinfectant solution. But this is not consistent, and I would hate to depend on it as an unhairing process. I think it may play a role in causing some of these inconsistencies that we are experiencing.

As to the leather quality, we have produced good vegetable leather. We have not as yet produced satisfactory chrome leather. That is a project we intend to work on. We don't know whether we can do it, but we hope we can. We feel that enzyme unhairing is sufficiently important to justify our spending effort to try and solve this problem.

DR. TURLEY: We hope to see you continue, Dr. Cordon, and solve this problem. I would like to call on one or two men here who have in the past had some experience with the use of enzymes in unhairing. Would Dr. Pfannmuller care to say a few words or ask a few questions?

DR. JULIUS PFANNMULLER (Wallerstein Company): I think Dr. Cordon's report was very encouraging in one way and in another way not. I think the really scientific approach would be to try to work with pure enzymes.

You can take crystallized enzymes, as for instance, crystalline trypsin or bacterial proteinases separated from amylases. You can get practically every enzyme either crystalline or in a concentrated, highly purified form, and such preparations might give you some important leads.

The difference in time of enzymatic hair loosening on thick pieces of hide versus goatskins might depend upon the time required for the enzyme to penetrate to the hair roots. This penetration has to occur especially from the flesh side. Of course, this penetration also depends on how the hide or skin was treated previously, that is, on whether the fiber structure is open and so gives the enzyme a better opportunity to penetrate to the roots.

You mentioned your experience of fine hair left over, which could not be removed properly by a mechanical unhairing machine. We have frequently observed that the fine hair after enzymatic loosening can be removed by drumming, because the hair is often loose. However, the mechanical unhairing rolls cannot grasp the hair.

I think we should also stress the economics of this process. Economic aspect plays a big role for the tanner, especially now. Highly concentrated enzyme preparations cost a lot of money, and while one should use them in laboratory experiments, they are too expensive for commercial use.

I think enzymatic unhairing is certainly a very fascinating problem, but it is far from being solved yet. That is my personal opinion, of course.

DR. CORDON: I agree one hundred percent. It is not solved. The enzymes are costly. We are trying to get at some of these fundamental problems in different ways—using purified products, using inhibitors, using different assay procedures to see if we can correlate an assay with hair loosening, and we feel that by gaining fundamental information of the mechanism of hair-loosening reaction we may learn just what is needed to remove the hair and do it more economically.

I think the enzyme companies could do a good deal if they would try to produce cheaper products. Many of these enzymes shown here are for food use. However, we do not need highly purified preparations to treat hides.

DR. PFANNMULLER: One more question. You mentioned a hide that you kept in a freezer for a long time. Did you make any microbiological tests about changes which might have occurred, or did you test skin sections microscopically as to what might have happened to the fiber structure?

DR. CORDON: No, we have not done this, but we could. We still have some of the material left.

DR. PFANNMULLER: I would be very much interested to know if the fiber bundles were broken.

DR. TURLEY: Would Dr. Thomas Thorstensen care to enter this discussion? He has done work in this field.

DR. THOMAS THORSTENSEN: I would like first to thank Dr. Cordon for his paper today. Dr. Cordon's work has been very helpful to us in the past, and I hope it will be continued. I agree, however, with Dr. Pfannmuller that the investigation of pure enzymes would be of great practical value in this field.

In the Tanners' Council beamhouse research project we have been working extensively with enzymatic unhairing. We have run numerous tests of various size on kid, calf, and cattle hides in several tanneries. Some of the problems Dr. Cordon mentioned are very real to us; others we feel have been solved.

A year ago, after Dr. Cordon's last paper on enzymatic unhairing, the remark was made, "It seems to be fashionable to work on enzymatic unhairing". In my opinion there is good reason for this to be the case. I think that enzymatic unhairing is much closer to being a commercially practical reality than most people here realize.

DR. TURLEY: Of course I think the big hurdle for all of these things is not the first stage—finding the enzyme to take off the hair—but to put it across and find out how to make good leather on a large scale. That is what demands a lot of work, and service too.

CLARENCE W. BEEBE (Eastern Regional Research Laboratory): I would like to say that I tanned that vegetable sole leather for Dr. Cordon. Dr. Turley asked why it made good vegetable leather but not good chrome leather. To obtain good vegetable leather required some adjustment of the pH to get the results that we did. So it is possible that the chrome leather, if adjusted properly, would also make good leather.

DR. TURLEY: When you adjusted the pH values, can you tell us how high you had to go?

MR. BEEBE: We went to pH 4. You could put it in lime first, if you wish, and then you could treat it just like an ordinary limed hide. But we adjusted to pH 4 and then used the rapid tanning process that has been developed and used in England, and with some success, by adjusting hides to pH 4 and tanning at pH 4.

DR. TURLEY: So it is unnecessary to use alkali?

MR. BEEBE: We did not have to use any alkali at all after we got rid of the calcium salts.

DR. TURLEY: That is very important, indeed.

MR. BEEBE: We were able to tan completely in 48 to 72 hours. We were able to penetrate completely.

DR. TURLEY: With straight vegetable tannins?

MR. BEEBE: Yes.

DR. TURLEY: That is a fine statement, Mr. Beebe.

DR. THEODORE D. BRAUNSCHWEIG (Loewengart and Company): I am interested as to whether the hides which you stored in the freezer have been stored open or packed?