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THE ACTION OF LIME SUSPENSIONS
ON STEER HIDE HAIR

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The Action of Lime Suspensions on Steer Hide Hair.

Among the many problems presented by the liming process those concerning the keratinous portions of the hides or skins are of considerable interest and importance. The hair shaft and root and the epidermis with its associated tissues comprise the bulk of such portions. Of these, the hair root and the epidermis are directly concerned in the unhairing action occurring during liming, and studies of the chemistry of unhairing should properly be made upon them. The difficulty of obtaining these tissues in sufficient quantity has, however, forced experimenters interested in this question to use the hair shaft in most of their investigations. The results of such studies are of much interest and value, yet in view of differences in composition and physical state of the tissues in question, their unqualified application to the behavior of the epidermis and hair root is hardly permissible.

Marriott and Merrill have especially studied the action of the ordinary depilatory solutions on hair. However, the greater portions of of their work deals with the action of sulfides and sulphhydrates. The effect of unsharpened calcium hydroxide suspensions on calf hair has been followed by Merrill⁽¹⁾ by means of nitrogen determinations.⁽²⁾ Marriott has considered

(1) H.B.Merrill and J.W.Fleming, Jr. Ind. Eng. Chem., 20,21(1928)

(2) R.H.Marriott, J.I.S.L.T.C., 12, 281 (1928);Ibid, 15,25(1931)

the source of the ammonia found in similar liquors.

A characteristic constituent of hair is the sulphur containing amino acid cystine. This amino acid, either alone or, as Bergmann has shown, more easily when in peptide combination, loses varying proportions of its sulphur as sulphide under the action of alkalies. The disulphide linkage of the cystine molecule ($\text{HOOC}\cdot\text{CH}(\text{NH}_2)\cdot\text{CH}_2\cdot\text{S}\cdot\text{S}\cdot\text{CH}_2\cdot\text{CHNH}_2\cdot\text{COOH}$) is also easily reduced to the sulphhydrate form ($\text{HOOC}\cdot\text{CH}(\text{NH}_2)\cdot\text{CH}_2\cdot\text{SH}$) of cysteine, a property shared by cystine peptides. The amino group of cystine and cysteine may be removed as ammonia under the action of alkalies. Cystine apparently retains this instability towards alkalies to a greater or lesser degree in its combinations in hair as, using the usual isolation procedure, Hoffman observed a marked decrease in the yield of cystine from hair previously treated with sodium carbonate solution. Marriott obtained similar results with sodium hydroxide, while Bergmann, W. Kuster and W. Irion and Marriott observe like behavior with sodium sulfide solutions.

While sulphides have been repeatedly found in old unsharpened lime liquors, yet, as far as we are aware, no quantitative studies of the various sulphur containing products formed during the normal liming period from the action of un-

(3) W.F.Hoffman, J.Biol.Chem. 65, 251-4 (1925).

(4) M.Bergmann and F.Stather, Collegium, 109(1925). Ibid. 249 (1926).

(5) W.Kuster and W. Irion, J.physiol.Chem., 184, 225 (1929).

(6) R.H.Marriott, J.S.L.T.C., 9, 612 (1925).

sharpened lime suspensions on hair have been published. Such a study would not only throw additional light on the behavior of the sulphur containing constituents of the hair during liming, but would also furnish a basis for the further study of the action of the various sharpening agents used in the beam house.

Experimental.

The ease of oxidation of the expected sulphur compounds demanded the comparison of two liming conditions (a) exposure to air and (b) as complete exclusion of air as possible. The latter condition was obtained by boiling the lime liquors before use, by "washing", the hair to be limed in a stream of nitrogen gas, and by carrying out the liming in an atmosphere of nitrogen gas in sealed bottles.

Throughout the liming process the liquors were agitated twice daily. At the end of the desired liming periods the liquors were filtered into nitrogen filled flasks while being protected from the action of atmospheric oxygen by a stream of nitrogen gas.

The limed hair was thoroughly washed as quickly as possible with unboiled distilled water before subjecting it to the desired analytical procedure.

Methods of analysis.

A. Analysis of the lime liquors.

1. Total Nitrogen.

Total nitrogen values were obtained on 25 c.c. portions of the filtered liquor by the Kjeldahl method.

2. Ammonia nitrogen.

Ammonia was determined by distillation under vacuum into excess acid.

3. Total sulphur.

A volumetric procedure was employed. The various forms of sulphur were oxidized to sulphate, which was determined by an iodometric procedure given by Kolthoff.⁽⁷⁾

4. Sulphur present as sulphide and sulphhydrate.

A procedure due to Kurtenacker and Wollack⁽⁸⁾ was used. The sulphide and sulphhydrate sulphur was calculated from the difference in iodine consumption of the lime liquor before and after treatment with $ZnCO_3$ suspension, which removes sulphide and sulphhydrate as zinc sulphide.

Results obtained by this procedure were satisfactorily checked by distilling H_2S from the acidified $ZnCO_3$ - ZnS precipitate obtained above into cadmium acetate solution and determining the cadmium sulphide iodometrically.

5. Sulphur present as sulphite.

Kurtenacker and Wollack's procedure was again used here. The result was calculated from the difference in iodine consumption of the liquor, (previously freed of sulphide and sul-

(7) M. Kolthoff. Volumetric Analysis, Vol. II, Practical Principles. p. 418.

(8) A. Kurtenacker and R. Wollack, Z. anorg. allgem. chem., 161,201 (1927); C.A., 21,1941 (1927).

hydrate by the $ZnCO_3$ suspension), before and after the addition of formaldehyde, which forms an aldehyde-sulphite compound not oxidized by iodine.

6. Sulphur present as polysulphide.

The estimation was based on a procedure due to E. Schulek which Bodnar and Gervay have found quite satisfactory. (9) By adding the sample of liquor to a boiling boric acid solution containing sodium cyanide polysulphide sulphur is converted to sulphocyanate which can then be estimated colorimetrically after the addition of ferric ion as the red ferric sulphocyanate. The conversion to sulphocyanate of the small amounts of polysulfide sulphur here dealt with is not as complete as obtained by Schulek for larger quantities. The polysulphide results recorded, while low, may still be considered comparative.

7. Sulphur present as sulphate.

Sulphur in this form was precipitated from the acidified liquor by the addition of an alcoholic solution of benzidine. The precipitate of benzidine sulphate was filtered off, washed and titrated with .01 N NaOH in the usual manner.

8. Sulphur present as cysteine and cystine.

(10)
Sullivan's method was utilized. A 50 c.c. portion of

(9) J. Bodnar and W. Gervay, Z.anal.Chem., 71,446 (1927).

(10) M. X. Sullivan, Public Health Reports, Supplement No. 78.

the lime liquor was prepared for analysis by freeing from sulphide and sulphhydrate with 5 c.c. of $ZnCO_3$ suspension. To a 50 c.c. portion of the supernatant liquid (obtained by centrifuging), acidified with 1 N HCl, 10 c.c. of 1 N HCl were added. The mixture was then diluted to 100 c.c., and examined for cysteine and cystine by Sullivan's procedure. This method utilizes the red color developed by the interaction of B-napthoquinone-p-sodium sulphonate and cysteine in the presence of sodium sulphite for the colorimetric determination of cysteine. Cystine is determined by this method by first reducing it to cysteine with sodium cyanide. Sullivan has shown the procedure to be highly specific.

9. Sulphur present as cysteine and cystine peptides.

A mixture of 25 c.c. of the liquor and 25 c.c. of concentrated HCl was boiled under a reflux for 10 hours, no attempt being made to exclude atmospheric oxygen. The hydrolysate, after concentrating to 10 - 15 c.c., was made alkaline to brom phenol blue by the addition of 6 N NaOH, diluted to 50 c.c., and examined for cystine by Sullivan's method.

Methods for the analysis of the limed hair.

1. Hydrolysis.

The limed hair, after a thorough but rapid washing with distilled water, was placed in a nitrogen filled flask and

(11). M. X. Sullivan, Public Health Reports, 41,1030 (1926).
Ibid., 44,1421 (1929). Ibid., 44,1599
(1929).

connected (by a ground glass joint) to a nitrogen filled reflux condenser. With a slow stream of nitrogen gas passing through the apparatus 50 c.c. of freshly boiled 20% HCl was introduced into the flask through the top of the condenser. Hydrolysis was effected by boiling the contents of the flask for ten hours. Hydrogen sulphide given off during the hydrolysis was collected in a cadmium acetate solution which also served as a seal to exclude atmospheric oxygen from the hydrolysate.

The final hydrolysate was concentrated to 20 - 25 c.c., cooled, and diluted with distilled water to 100 c.c.

2. Total nitrogen.

A 10 c.c. aliquot of the hydrolysate was analysed for total nitrogen by the Kjeldahl.

3. Ammonia nitrogen.

A 25 c.c. aliquot of the hydrolysate was added to 100 c.c. of water containing an excess of Ca(OH)_2 and the mixture distilled, under vacuum, into excess standard acid.

4. Total sulphur.

This was determined in a 10 c.c. portion of the hydrolysate by the procedure mentioned under the analysis of the lime liquors.

5. Sulphur given off as hydrogen sulphide during hydrolysis.

The cadmium sulphide formed in the cadmium acetate solution mentioned above was determined iodometrically.

6. Sulphur present as cysteine and cystine.

(12)

These values were obtained by Okuda's procedure, which depends upon the oxidation of cysteine in dilute hydrochloric acid solution containing potassium iodide by the addition of standard potassium iodate solution. Cystine is determined by this procedure after reduction to cysteine with zinc and hydrochloric acid.

Results.

In Table I is recorded the analytical results for lime liquors obtained by liming, at 18^o C., six gram portions of hair in 500 c.c. of saturated lime water containing 1.5 grams excess Ca(OH)_2 per 100 c.c. of solution. The hair used in this experiment had been clipped from a fresh steer hide, washed and air dried. It had not been treated with fat solvents. These lime liquors were not examined for cysteine or cystine, nor was the limed hair analysed. The nitrogen gas used in this experiment was not freed of the small amount of oxygen it contained.

To enable ready comparison of the quantities of the various forms of sulphur present, all results have been expressed as milligram atoms (one thousandth of a gram atom) per gram of unlimed hair.

The irregular variation of the total nitrogen results is very marked. This may possibly be due to uneven fat distribu-

(12) Y. Okuda, J. Sci. Agr. Soc. (Japan) No. 253, 1-3 (1923);
S. A. 18, 2014 (1924).

Action of Ca(OH)₂ Suspensions at 16°C. (64°F.) on Hair from a Fresh Steer Hide.

Analysis of Final Lime Liquors.

Milligram atoms in liquor per gram of hair.

Days Liming of	Limed in presence of	Total Nitrogen	Ammonia Nitrogen	Sulphide plus sulph- hydrate sulphur	Poly- sulphide sulphur	Sulphite sulphur	Sulphate sulphur	Total sulphur	% of Total sulphur in liquor accounted for.	% of Tot- al sul- phur of hair found in lime liquor.
1	Air	.348	.068	.004	.004	.007	.009	.107	22.4	8.8
1	Nitrogen	.994	.051	.000	.020	.005	.003	.127	23.0	10.5
2	Air	.190	.064	.005	.012	.007	.016	.178	22.0	14.7
2	Nitrogen	1.145	.060	.014	.028	.002	.004	.162	29.9	13.4
3	Air	.239	.071	.003	.023	.009	.040	.241	31.1	19.9
3	Nitrogen	1.331	.071	.014	.041	.001	.010	.200	33.3	16.5
5	Air	.294	.108	.005	.024	.009	.059	.281	34.5	23.2
5	Nitrogen	.564	.096	.023	.069	.000	.010	.234	44.1	19.3
8	Air	.457	.131	.009	.021	.008	.079	.324	36.1	26.8
8	Nitrogen	.494	-	.037	.078	.000	.017	.287	46.0	23.7
13	Air	.911	.194	.009	.016	.006	.124	.406	38.2	33.5
13	Nitrogen	1.035	.200	.047	.094	.000	.015	.338	46.1	27.9

tion, as Marriott has noticed marked differences in the nitrogen content of sodium sulphide solutions which had acted upon degreased and undegreased goat hair. The ammonia results do not show this irregularity, but increase with the time of liming and with no significant difference between the two liming conditions of exposure to and exclusion of air.

The effect of these conditions is well demonstrated by the sulphur distribution. The lower states of oxidation (sulphide plus sulphhydrate and polysulphide) are much more abundant in those liquors obtained with the exclusion of air than in those to which atmospheric oxygen has had access. The reverse is found in the case of sulphur present as sulphate. Sulphite sulphur hardly exceeds experimental error at any time, but runs higher in those liquors exposed to air.

The total sulphur content of the liquors increases with the time of liming. The increase is quite rapid during the first two days. Except for the one day liming period, less sulphur is determined in the unexposed liquors than in those in contact with air. This may reflect a difference in the behavior of hair under the two liming conditions employed, possibly effected through the differences in sulphur distribution.

However, at the end of a normal liming period of five days, only about 40% of the sulphur compounds in the lime liquor have been accounted for. At this time the lime liquor has removed from hair approximately 20% of its sulphur.

In Table II is recorded the changes occurring in the sulphur distribution of the 8 day liquors, obtained in the above experiment on allowing to stand, after the removal of the hair, under the noted conditions. The liquor obtained with the exclusion of air seems to be quite stable when kept under these conditions, but, on exposure to air, suffers a loss of sulphide plus sulphhydrate and polysulphide sulphur with a gain in the sulphite and sulphate forms. This is naturally to be expected, though it is interesting to note that this gain does not equal the loss of sulphide, sulphhydrate and polysulphide observed, as is seen on comparing the summation of the various forms of sulphur determined. This difference may be due to the formation of thiosulfate or other substances not determined in the present analysis.

In Tables III, IV and V are gathered results of a similar experiment performed on hair clipped from a Domestic steer hide. This hair had been washed, air dried, extracted with five or six portions of benzene at room temperature for seven or eight days and finally air dried. Complete fat extraction was probably not obtained by this treatment.

The nitrogen gas used to exclude atmospheric oxygen was freed of oxygen by bubbling through three Truog towers containing alkaline pyrogallol (prepared according to Anderson) and

(13) Truog, J. Ind. Eng. Chem., 7, 1045 (1915).

(14) Anderson, J. Ind. Eng. Chem., 8, 131, 133 (1916).

Table II.

Post Liming Changes in Sulphur Distribution of Lime Liquors.

Results expressed as milligram atoms of sulphur in the lime liquor per gram of hair.

Days in limes	Limed in presence of	Post Liming conditions	Sulphide plus sulph- hydrate sulphur	Poly- sulphide sulphur	Sulphite sulphur	Sulphate sulphur	Summation
			a.	b.	c.	d.	a+b+c+d
8	Air	Immediate analysis of liquor.	.009	.020	.009	.080	.118
		Analysis of liquor after standing exposed to air for 24 hours.	.008	.009	.014	.084	.115
8	Nitrogen	Immediate analysis of liquor.	.037	.078	.000	.017	.132
		Analysis of liquor after standing exposed to air for 19 hours.	.006	.005	.024	.037	.072
		Analysis of liquor after standing protected from air for 19 hours.	.035	.068	.002	.011	.116

then over heated copper gauze, a procedure recommended by Andrews.⁽¹⁵⁾

The determination of sulphite in the lime liquors was omitted, but cysteine, cystine and their polypeptides were tested for by the methods described previously. Sulphur present in the liquor as sulphide and sulphhydrate was determined by distillation of the $ZnCO_3$ - ZnS precipitate, as outlined above, instead of by iodometric titrations. The limed hair from this experiment was also examined by the procedure given.

Table III presents the analyses of the final lime liquors. The total and ammonia nitrogen results are still probably questionable, and, in the absence of a more extended series of determinations, will not receive serious consideration here.

The total sulphur content and its distribution agree with that shown in Table I. The curing and partial defatting undergone by the Domestic steer hide hair hence does not greatly influence its sulphur losses in lime suspensions.

In no case was cysteine or cystine found, either as the amino acid or in peptide combination. As Sullivan's test is highly specific this finding does not exclude the presence of other organic sulphur compounds. No substances interfering with the Sullivan method were present in the acid hydrolysed lime liquor as was shown by the satisfactory determination of a known amount of added cystine.

(15) J. C. Andrews, J. Biol. Chem., 80, 191 (1928).

Table III.

Action of Ca(OH)₂ Suspensions at 20°C. (68°F.) on Hair from a Domestic Cured Steer Hide.

Analysis of Final Lime Liquors.

Milligram atoms in liquor per gram of unlimed hair.

Days Liming	Limed in presence of	Total Nitrogen	Ammonia Nitrogen	Sulphide plus sulphate sulphur	Poly-sulphide sulphur	Sulphate sulphur	Total sulphur	Cysteine sulphur	Cystine sulphur	Cysteine or cystine polypeptide sulphur
4	Air	.251	.053	.002	-	.046	.254	none	none	none
4	Nitrogen	.204	.052	.012	-	.004	.230	none	none	none
5	Air	.247	.089	.004	.012	.072	.276	none	none	none
5	Nitrogen	.191	.071	.018	.077	.006	.241	none	none	none

Table IV shows the results obtained on the hydrolysates of the limed and unlimed hair. The H_2S given off during hydrolysis amounts to only 1% of the total sulphur present, but is higher for limed than for unlimed hair. Hair limed in the absence of air yields slightly more H_2S than when limed in its presence. These values may possibly reflect different degrees of chemical changes occurring in the hair under the action of the different liming conditions.

The cysteine sulphur does not vary significantly with the liming conditions employed or from that found for unlimed hair.

The cystine analysis clearly reflect the action of the lime suspension. Only fifty percent of the cystine determined in unlimed hair remains after four or five days liming. Examination of the data shows, however, that the cystine and cysteine determinations account for only about 50% of the total sulphur content of the limed hair.

Table V summarizes the data presented in Tables III and IV. The recovery of total nitrogen and total sulphur as shown by their corresponding summations is not entirely satisfactory. A maximum loss of 8% of the nitrogen and 12% of the sulphur (ignoring the questionable value) occurred during the manipulations required. Only 50% of the total sulphur of the unlimed hair can be accounted for after five days liming, at which time the remaining cystine sulphur constitutes only 35 to 37% of the total sulphur content of the unlimed hair.

Action of Ca(OH)₂ Suspensions at 20°C. (68°F.) on Hair from a Domestic

Cured Steer Hide.

Analysis of Limed Hair.

Milligram atoms in limed hair per gram of unlimed hair.

Days Liming	Limed in presence of	Total Nitrogen	Ammonia Nitrogen (formed on acid hydrolysis)	Total sulphur	Sulphur evolved as H ₂ S during the acid hydrolysis	Cysteine sulphur	Cystine sulphur
	Unlimed, un- hydrolysed hair	10.30		1.360			
	Unlimed, hydrolysed hair	10.07	.753	1.042	.003	.072	.918
4	Air	9.57	.763	.718 (?)	.005	.063	.584
4	Nitrogen	9.83	.846	1.107	.010	.063	.477
5	Air	9.28	.733	.987	.007	.066	.439
5	Nitrogen	9.63	.724	.945	.009	.069	.442

Action of Ca(OH)₂ Suspensions at 20°C. (68°F) on Hair from a Domestic

Cured Steer Hide.

Summary of Data in Tables III and IV.

Milligram atoms per gram unlimed hair

Days Liming	Limed in presence of	Σ total nitrogen in liquor and limed hair	Σ ammonia nitrogen in liquor and limed hair	Σ total sulphur in liq- uor and limed hair	Σ sulphur accounted for in liquor and limed hair	% of total sulphur accounted for	Cysteine sulphur in limed hair as % of total sulphur	Cystine sulphur in limed hair as % of total sulphur
4	Air	9.82	.816	.977 (?)				
4	Nitrogen	10.04	.898	1.347			4.7	35.4
5	Air	9.53	.822	1.270	.600	47.2	5.2	34.6
5	Nitrogen	9.82	.795	1.195	.600	50.2	5.8	37.0
Unlimed, hydrolysed hair		10.07	.753	1.045			6.9	88.1
Unlimed, un- hydrolysed hair		10.30		1.360				

Summary.

The action of unsharpened lime suspensions on steer hide hair, with particular reference to the sulphur compounds obtained, has been studied.

Exposure to or exclusion of atmospheric oxygen during liming produces marked differences in the state of oxidation of some of the inorganic sulphur compounds formed.

The data obtained shows no other marked differences due to these liming conditions.

The formation of polysulphide sulphur in the absence of atmospheric oxygen indicates that some other substance present is capable of oxidizing the sulphides formed during liming.

No cysteine or cystine, either as amino acid or in peptide combination, is present in the liquors examined.

The cystine content of the hair is decreased 50% by five days liming, the cysteine content remains quite constant.

Much of the sulphur remaining in the limed hair is not present as cysteine or cystine compounds.