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## THE ACTION OF ACRYLONITRILE AND ACRYLAMIDE UPON COLLAGEN

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### ABSTRACT

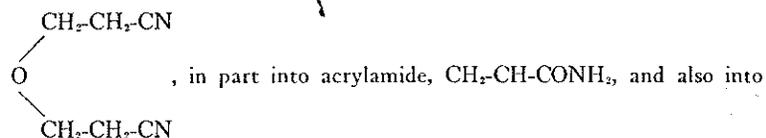
Acrylonitrile and acrylamide are shown to react with limed hide in strongly alkaline solutions. The fixation of the monomers under such conditions results in a lowering of the shrinkage temperature and in a decreased chrome fixation. The dry fiber strength of the resulting leather is not materially affected. The  $\epsilon$ -amino groups are considered to be the active sites in the cyano- and carbamoylethylation of collagen.

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### INTRODUCTION

In caustic solutions cellulosic materials can be cyanoethylated without difficulty. Proteins lack the necessary resistance toward strong alkalis and cannot be treated in the same manner. Recently, however, wool has been cyanoethylated under relatively mild conditions (1). The wool was first soaked at room temperature in a solution of 4 g/l or less NaOH and then treated with a large excess of acrylonitrile at 60°C. In the cyanoethylation of cotton the reaction temperatures usually range from 65° to 85°C. A recent pilot-plant experiment (2) demonstrated that the nitrogen content of yarn after cyanoethylation at less than 40°C. is not significantly lower than after a similar treatment at the usual temperatures. This re-evaluation of the role temperature plays in cyanoethylation processes seems to the authors to open up possibilities of reacting acrylonitrile with collagen.

Acrylonitrile is unstable in alkaline solutions. In the presence of water it converts mostly into  $\beta, \beta'$ -oxydipropionitrile (bis-2-cyanoethylether),



sodium acrylate (2). Only acrylamide, which is now available as a pure crystalline product, is still capable of reacting with cellulosic materials. Acryla-

mide combines with cotton at 50°C. in precisely the same ratio of 1 mole for 5.7 anhydroglucose units which prevailed in the cyanoethylation of yarn below 40°C. (3). It may be assumed, therefore, that both acrylonitrile and acrylamide can be introduced with equal ease into the collagen molecule. How attempts to prove this succeeded is described in the present paper.

New modifications of collagen may prove to be feasible for extending its usefulness as in the case of other materials. In the case of cotton, for example, cyanoethylation has been found effective in improving its dyeability and increasing its rot resistance. The present study, exploratory as it necessarily is, may point the way toward similar improvements. Such a possibility is suggested by the observation that the formaldehyde fixation can be increased by the reaction with acrylamide.

#### EXPERIMENTAL

Techniques similar to those employed in a recent study on aldehydes (4) were used to overcome the swelling effect of alkaline solutions upon collagen. Either a so-called alkaline pickle was employed, i.e., the  $\text{Na}_2\text{SO}_4/\text{NaOH}$  system which alone leaves hide flaccid at pH values beyond 11, or NaOH was added to 2*N*  $\text{Na}_2\text{CO}_3$  solutions which also have no plumping action. The  $\text{Na}_2\text{CO}_3/\text{NaOH}$  system does not cause the hides to become as flaccid as in the  $\text{Na}_2\text{SO}_4/\text{NaOH}$  system. A few experiments were conducted in the absence of caustic soda in order to investigate the reactivity of acrylonitrile or acrylamide at lower pH values.

For most experiments pickled cow grains were used. Only the first experiment was conducted with pickled steer bends. Pieces weighing 50 to 350 g., selected from opposite locations on two sides of a single hide, were tumbled sufficiently long to assure equilibrium with the alkaline solutions before introducing the monomers. In their presence agitation continued 2 hr. for the cow grains and 5 hr. for the steer bends.

Thereafter, in the case of the cow grains, the liquor pH was gradually lowered to approximately 4.5 by the addition of formic acid. At the outset the intervals between feeds of the acid were 10 to 15 minutes; they were lengthened when the pH of the solution approached the desired range. In the  $\text{Na}_2\text{CO}_3/\text{NaOH}$  system as much as 15%, on the pickled weight, of 90% formic acid was needed for reaching the desired pH. In the  $\text{Na}_2\text{SO}_4/\text{NaOH}$  system between 2.5 and 3% was required.

In the case of the steer bends the liquor was drained off before the first portion of formic acid was added. Only the  $\text{Na}_2\text{CO}_3/\text{NaOH}$  system was investigated and because only the alkali inside the hide pieces remained to be neutralized, the acid consumption was 2/3 lower than for the cow grains.

Finally all pieces were washed in running tap water as well as in several changes of distilled water; most were subsequently chrome tanned. Small

strips were air-dried. Only pieces of the steer bend, in the first test, were tanned with formaldehyde by adding 5%, on pickled weight, of a commercial solution directly to the alkaline liquor. Assuming 20% of the pickled pelt to be collagen, this quantity equals 9 parts  $\text{H}_2\text{CO}$  for 100 parts or 3 millimoles per 1 g. collagen. This ratio was selected because it had given yields of about 20% in a recent study (4).

The chrome tannage follows, on the whole, Gustavson's technique (5) which purposely employs a very large excess of chrome in order to measure the maximum capacity of collagen or modified collagen for its fixation.

In Gustavson's experiments a 33% basic chromic sulfate liquor represented the cationic type, while a glucose-reduced liquor containing 2.5 moles  $\text{Na}_2\text{SO}_4$  per mole chromic oxide served as the example of a chrome liquor containing mostly noncationic chrome. The cationic chrome liquor in our study was a 33% basic commercial chrome tanning material, boiled and aged as a solution containing 100 g/l  $\text{Cr}_2\text{O}_3$ . By using 200 volume % of this solution, the ratio of  $\text{Cr}_2\text{O}_3$  to pelt was 1 to 1. When the noncationic chrome liquor was prepared according to Gustavson's directions, it jelled while aging. In a second solution a pH of 5.8 was found a few hours after preparation. Therefore 0.13 moles  $\text{H}_2\text{SO}_4$  per mole  $\text{Cr}_2\text{O}_3$  were added, which lowered the pH to 3.7; within 2 weeks the pH gradually rose again, this time to 4.6, necessitating once more the addition of 0.065 moles  $\text{H}_2\text{SO}_4$  per mole  $\text{Cr}_2\text{O}_3$ . On the day when the solution was used its pH was 4.2. Since it contained approximately 20 g/l  $\text{Cr}_2\text{O}_3$ , it was necessary to apply 1000 volume % in order to reach the same ratio for  $\text{Cr}_2\text{O}_3$  as in the cationic liquor.

The tannage with the cationic liquor took place at pH 3.3 preceded by a mild pickle in an  $\text{H}_2\text{SO}_4/\text{NaCl}$  system. During tannage, a total of 0.16 moles soda ash per mole  $\text{Cr}_2\text{O}_3$  was added in small feeds over a period of 6 hr. to promote the chrome pickup.

The second tannage was not preceded by a pickle nor did it require the addition of soda ash. The milling time, however, was the same as in the first one.

After the tanned hide pieces were kept in the wet stage for 16 hr., they were thoroughly washed and air-dried.

#### ANALYTICAL METHODS

**Physical.**—The shrinkage temperatures were determined according to standard techniques except in the case of chrome pieces which were thought to shrink over 100°. These measurements were made with the Transverse Shrinkage Meter (6). Burst measurements were conducted on a Scott Tester with the 1/8-inch plunger (7).

**Chemical.**—Cyanoethylated products are, as a rule, assayed by the Kjeldahl method. Since they usually are free, or nearly free, of nitrogen

before the cyanoethylation, a direct digestion is, without doubt, the most reliable analytical procedure. In fact, a warning has recently been issued against the practice, apparently wide spread, of omitting the digestion with  $H_2SO_4$  (8): the direct distillation of the  $NH_3$  formed on boiling cyanoethylated cotton with dilute NaOH at best yields only 95% of the nitrogen. Methods based on the digestion of acrylonitrile- or acrylamide-treated raw hide would probably be no more precise since the correct distribution of the nitrogen between collagen and monomers could only be found if the balance of all constituents (e.g., ash, grease) as well as the nitrogen factor was accurately known.

The direct distillation, on the other hand, yields excess  $NH_3$  because dilute alkali hydrolyzes collagen (9). After tanning pickled cowhide in the manner described above for maximum chrome pickup, the volatile nitrogen was about 6% of the total nitrogen for the cationic and noncationic tannages alike. These figures were applied as correction values to the results obtained by hydrolyzing in the same manner chrome-tanned modified hide. The distillation was improved by conducting it in an apparatus developed for  $H_2CO$  determinations in leather (10). One gram of ground leather was hydrolyzed by boiling it with 100 ml. of a 0.6*N* NaOH solution. After the first 20 ml. were distilled over, the volume was maintained by slowly adding the water needed until 2 fractions of 100 ml. each had been collected and summarily titrated. A third fraction contained no more than 0.05 meq.  $NH_3$  and could, therefore, be dispensed with in the controls as in the treated specimens.

The result of the titration was a direct measure of the quantities of bound acrylonitrile or acrylamide. The acid consumed was also subtracted from the total acid consumption in a standard Kjeldahl determination in order to obtain true hide substance values. The factors for the conversion of nitrogen into acrylonitrile and acrylamide are 3.8 and 5.0 respectively.

Chrome was determined by wet oxidation. The formula of Kanagy (11) was used to arrive at a figure for the chrome complex in both the cationic and the noncationic tannages, although its validity has only been proven for the former tannage.

## RESULTS

**Treatments.**—Substitutions of specific groups do not qualify as tannages since they do not cause cross linking. In fact, many instances are known where they may even effectively block the reaction with selective tanning agents. In these cases significant decreases in shrinkage temperature have been observed. In our experiments with acrylonitrile and acrylamide therefore, observations of the  $T_s$  changes were the first indications of a reaction taking place between these monomers and the collagen.

TABLE I  
SHRINKAGE RESISTANCE OF STEERHIDE TREATED WITH  
ACRYLONITRILE OR ACRYLAMIDE WITH OR WITHOUT A  
FORMALDEHYDE AFTERTREATMENT

(Pickled steer bends\*, in 50 volume percent† solution of 240 g/l  $Na_2CO_3$  and 50 g/l NaOH)

	Acrylonitrile	Acrylamide
% monomer†	6	7
$T_s$ , °C., at pH 11.8, 2 hr. later	52	55
pH lowered with formic acid to	5.6	5.7
$T_s$ , °C., after lowering pH and subsequent washing:		
a) for $H_2CO$ -treated pieces	64	72
b) for pieces not treated with $H_2CO$	50	50

\*Pieces paired off as required on the basis of bilateral symmetry.  
†On pickled weight.

Table I presents these observations in tests with pieces of steerhide which have been more closely described above. When the treatment with acrylonitrile or acrylamide preceded a formaldehyde tannage, the pieces shrank 28° and 20° C. respectively lower than without it. This statement is based on the recent observation that under comparable conditions a formaldehyde-tanned steerhide shrinks at 92° C. (4), or 29° higher than the same hide untanned\*. By comparison the rise after carbamoylethylation was only 22° C. and after cyanoethylation, 14° C.

The next set of experiments had the purpose of ascertaining whether or not NaOH is indispensable for reacting the monomers with collagen. In the cyanoethylation of cotton and wool caustic soda is always used. The new experiment employed either soda ash in the same concentration as the previous one, which was approximately 2*N* after equilibration with the hide pieces, or, as the example of a milder alkali sodium sesquicarbonate for which a final concentration of about 1.5*N* was selected. The quantity of monomers employed was approximately double in order to insure a reaction if one was at all possible under the new conditions. Further details are presented in Table II together with the  $T_s$  values obtained at the same two stages as in Table I. The table shows that the highest pH was 9.8 as against 11.8 in presence of NaOH and that only acrylonitrile is still capable of materially lowering the  $T_s$  of hide under these circumstances. Acrylamide displays a stronger lyotropic effect (12*b*) in the alkaline range than before, simply because its concentration was raised from 0.8 to more than 1.8 moles/l. A piece of hide shrank at 47° C. when removed from the acrylamide  $Na_2CO_3$  solution of pH 9.8, but another piece taken later shrank 18° C. higher when free of electrolytes.

\*Gustavson (12*a*) records a slightly higher increase of 32°C.

TABLE II  
SHRINKAGE RESISTANCE OF COWHIDE TREATED WITH  
ACRYLONITRILE OR ACRYLAMIDE IN NONCAUSTIC  
ALKALINE SOLUTIONS

(Pickled cow grains, in 50 volume percent\* solution)

	Acrylonitrile		Acrylamide	
% Na <sub>2</sub> CO <sub>3</sub> *	12.5	—	12.5	—
% Na <sub>2</sub> CO <sub>3</sub> ·NaHCO <sub>3</sub> ·2H <sub>2</sub> O*	—	15.0	—	15.0
% monomer*	12.0	12.0	16.0	16.0
pH, 90 minutes later	9.7	9.5	9.8	9.4
pH lowered with formic acid to	4.1	3.8	4.1	3.9
T <sub>s</sub> , °C., before lowering the pH	51	54	47	53
T <sub>s</sub> , °C., after lowering the pH and subsequent washing	53	55	65	58

\*On pickled weight.

TABLE III  
SHRINKAGE RESISTANCE OF COWHIDE TREATED WITH  
ACRYLONITRILE OR ACRYLAMIDE IN SWELLING-DEPRESSING  
CAUSTIC SOLUTIONS

(Pickled cow grains in 50 volume percent\* solution)

	Acrylonitrile		Acrylamide		Control	
% Na <sub>2</sub> CO <sub>3</sub> *	12.0	—	12.0	—	12.0	—
% Na <sub>2</sub> SO <sub>4</sub> *	—	12.5	—	12.5	—	12.5
% NaOH*	3.1	3.0	3.1	3.0	3.1	3.0
% monomer*	12.0	12.0	16.0	16.0	—	—
pH lowered to	5.0	5.1	4.9	4.8	4.1	4.0
% formic acid (90%) added for lowering pH	15.0	2.5	15.0	1.3	9.5	1.6
T <sub>s</sub> , °C., before lowering pH	55	54	43	43	59	63
T <sub>s</sub> , °C., after lowering pH and subsequent washing	49	49	52	52	63	63

\*On pickled weight.

An experiment designed for a comparison between the "alkaline pickle" and a solution of caustic alkali in 2*N* soda ash is recorded in Table III. In both instances the quantity of sodium hydroxide was about the same. However, acrylamide, for which this effect was already considerable in the absence of NaOH (Table II), now caused a T<sub>s</sub> depression of 20° as against 16° before.

In the case of acrylonitrile the lyotropic effect is probably caused by secondary products and is much less pronounced, although somewhat higher than in the "Controls" which contain no monomers at all. The permanent T<sub>s</sub> losses are 14° C. for the cyanoethylated and 11° C. for the carbamoyl-ethylated hide, regardless of the alkaline bath used. These decreases are not very different from those obtained on much heavier hide with about half of the monomer concentration (Table I). Yet the denser fiber structure of steer bends seemed to obscure the greater T<sub>s</sub> depressing effect of acrylonitrile, at least before a retannage was applied, whereas grain splits show the difference without it.

All hide pieces dried out as horny\* and stiff as raw hide with a brownish color. In the wet stage their color depended less on the monomer than on the alkaline system employed. Still, the modified hide pieces could be distinguished from pickled ones of the same hide which had not been modified. Table IV attempts to describe the color hues observed. Evidently the grain was less prone to color changes than the flesh side.

TABLE IV  
COLOR CHARACTERISTICS OF COWHIDE  
AFTER TREATMENT WITH ACRYLONITRILE OR ACRYLAMIDE  
(In the wet stage after de-alkalizing and rinsing)

	Alkaline System			
	Na <sub>2</sub> SO <sub>4</sub> /NaOH		Na <sub>2</sub> CO <sub>3</sub> /NaOH	
	Grain	Flesh	Grain	Flesh
1) Untreated.	grayish white	light purple	grayish white	light purple
2) Acrylonitrile-treated	purplish	grayish white	grayish white	dark purple
3) Acrylamide-treated	grayer than (1)	lighter purple than (1)	grayish white	dark purple

**Examination of chrome leathers.**—Treated pieces and controls of Table III, after being chrome tanned as described above, were tested for thickness, strength, and shrinkage resistance (T<sub>s</sub>). The figures obtained for the first two of these properties are compiled in Table V. The T<sub>s</sub> values will be presented separately in an attempt to link them with the chrome content. A final tabulation will disclose the finding of the chemical analysis.

**Strength properties.**—The burst measurements, as recorded in Table V, allow one important conclusion: The strength of the leathers was not affected by reacting the hide with the two monomers before tanning. The only significant difference in strength which can be observed on untreated

TABLE V

THICKNESS AND STRENGTH CHARACTERISTICS OF  
CHROME-TANNED LEATHER MADE FROM  
CYANOETHYLATED OR CARBAMOYLETHYLATED COWHIDE

	Acrylonitrile		Acrylamide		Controls	
	Na <sub>2</sub> CO <sub>3</sub> / NaOH	Na <sub>2</sub> SO <sub>4</sub> / NaOH	Na <sub>2</sub> CO <sub>3</sub> / NaOH	Na <sub>2</sub> SO <sub>4</sub> / NaOH	Na <sub>2</sub> CO <sub>3</sub> / NaOH	Na <sub>2</sub> SO <sub>4</sub> / NaOH
<i>Thickness, in.</i>						
Cationic tannage	0.090	0.085	0.092	0.091	0.079	0.096
Noncationic tannage	0.101	0.097	0.094	0.102	0.091	0.094
<i>Bursting load, lb.*</i>						
Cationic tannage	53	43	50	42	33	38
Noncationic tannage	72	62	65	41	48	61
<i>Bursting strength, lb/in</i>						
Cationic tannage	590	500	550	460	420	400
Noncationic tannage	710	640	690	390	530	640

\*Avg. of 8 determinations.

TABLE VI

CHROME CONTENTS AND SHRINKAGE TEMPERATURES OF CHROME-TANNED LEATHER MADE FROM CYANOETHYLATED OR CARBAMOYLETHYLATED COWHIDE

	Acrylonitrile		Acrylamide		Controls	
	Na <sub>2</sub> CO <sub>3</sub> / NaOH	Na <sub>2</sub> SO <sub>4</sub> / NaOH	Na <sub>2</sub> CO <sub>3</sub> / NaOH	Na <sub>2</sub> SO <sub>4</sub> / NaOH	Na <sub>2</sub> CO <sub>3</sub> / NaOH	Na <sub>2</sub> SO <sub>4</sub> / NaOH
<i>Chromic oxide, percent on hide substance basis</i>						
a. Cationic tannage	9.3	8.9	10.3	10.3	11.6	11.6
b. Noncationic tannage	11.1	11.2	14.9	22.1	17.5	16.0
Difference b - a	1.8	2.3	4.6	—	5.9	4.4
<i>T<sub>s</sub>, °C., (water only, under pressure)</i>						
a. Cationic tannage	102	102	112	110	118	116
b. Noncationic tannage	96	98	111	109	115	113
Difference a - b	6	4	1	1	3	3
<i>T<sub>s</sub>, °C., (glycerin only):</i>						
a. Cationic tannage	111	110	121	120	133	128
b. Noncationic tannage	109	111	121	121	133	135
Difference a - b	2	-1	0	-1	0	-7
<i>T<sub>s</sub>, °C., increase in glycerin:</i>						
a. Cationic tannage	9	8	9	10	15	12
b. Noncationic tannage	13	13	10	12	18	22

and treated leather pieces alike is connected with the tannage. The non-cationic sulfite-chrome liquor gives stronger leather. This is the more remarkable since this liquor causes more chrome to be fixed (Table VI). The same liquor produces a somewhat fuller leather. The increase in thickness averages about 8% for the whole set of specimens. Consequently the increase in bursting strength is smaller than that in bursting load, namely 20% versus 26%. For the untreated controls the increases are 30% and 35% respectively. These percentages are based on the original measurements, not on the rounded figures of Table V. The alkaline systems also seemed to exert a distinct influence on the strength properties when the test pieces were selected for them on the principle of bilateral symmetry. This was, however, not the case with the controls because of a dearth of specimens. The "alkaline pickle" lowered the bursting load of the leather by about 22%, regardless of the chrome liquor in which it was tanned. The bursting strength was also 15 to 20% lower; here the loss was greater for cationically tanned leather which as a whole turned out to be inferior in strength to the sulfite-chrome-tanned hide pieces.

**Visual and tactile inspection.**—None of the leathers were fatliquored before being tested. Those tanned with cationic chrome were generally more pliable and less tender on the grain because of their lower chrome content (Table VI). By reacting with the monomers the leather became softer and more flexible; the pieces treated in presence of soda ash felt drier and stiffer, presumably because they retain less natural grease. A pronounced greenish cast was typical of the sulfite-chrome tannage, while the cationic liquors produced the usual bluish-green color.

**Relationship of chrome pickup to shrinkage resistance.**—In Table VI chrome contents are shown in juxtaposition with shrinkage temperatures obtained in glycerin and in water. In appraising the figures presented in this table one notes the following: (a) The alkaline systems employed do not influence chrome fixation or *T<sub>s</sub>*. (b) By a noncationic tannage more chrome is fixed than by a cationic tannage, while the *T<sub>s</sub>* is slightly lowered. (c) After reacting with acrylonitrile hide picks up less chrome than it would normally, but after reacting with acrylamide it fixes either the same amount or even more. However, it is quite possible that the single figure of 22.1% Cr<sub>2</sub>O<sub>3</sub>, representing the highest fixation in the table, is the combined result of a tannage and an excessive aggregation of the sulfite-chrome complex leading to a high interfibrillary deposition. Gustavson also recorded inordinately high chrome contents in sulfite-chrome leather (14), without, however, considering the possibility of some of the chrome being held by mechanical forces after aggregation has progressed beyond a certain point. (d) The *T<sub>s</sub>* is lowered by the attachment of monomers to the hide substance, more so with acrylonitrile than with acrylamide, and more by a noncationic

than by a cationic tannage. (c) In glycerin the  $T_s$  is higher than in water as may be expected from data linking it to the medium's dielectric constant (14b). The difference is larger for a sulfite-chrome tannage than for a cationic chrome tannage, and considerably larger without a treatment than after acrylamide or acrylonitrile. The  $T_s$  values by the ALCA method are not shown in Table VI because the controls did not shrink before the boiling point of the mixture. The cyanoethylated pieces shrank 5°–8° higher and the carbamoylethylated pieces 1°–3° lower than in water under pressure.

**Fixation of monomers and chrome complex.**—Determination in the leathers listed in Table III of hide substance, chrome complex according to Kanagy (11), and the two monomers, where present, gave the results of Table VII. Since natural grease or inorganic matter other than chrome was not determined, the sum total was not expected to approach 100%. Instead, in most cases, it was even higher than 100. In three instances where it exceeded 102, the figures for the chrome complex were also very high, and the nature of the tannage was noncationic. Under these conditions, apparently, Kanagy's formula is not valid, an observation which seems to confirm the idea expressed above that some chrome has aggregated and is no longer chemically bound to collagen. Table VII also shows that the fixation of acrylonitrile in millimoles/g. collagen is, on the average, about twice as

TABLE VII  
CHEMICAL COMPOSITION OF CHROME LEATHER  
FROM COWHIDES TREATED WITH ACRYLONITRILE OR ACRYLAMIDE

	NaOH Applied with	Acrylonitrile	Acrylamide	Percent on dry basis			Millimoles Monomer per g. Collagen
				Chrome Complex	Hide Subs.	Sum	
Cationic tannage							
	Na <sub>2</sub> CO <sub>3</sub>	—	—	21.9	78.8	100.7	—
	Na <sub>2</sub> SO <sub>4</sub>	—	—	21.9	79.5	101.4	—
	Na <sub>2</sub> CO <sub>3</sub>	5.6	—	18.1	78.1	101.8	1.4
	Na <sub>2</sub> SO <sub>4</sub>	4.8	—	17.4	79.0	101.2	1.1
	Na <sub>2</sub> CO <sub>3</sub>	—	4.1	18.9	74.6	96.7	0.8
	Na <sub>2</sub> SO <sub>4</sub>	—	4.1	18.7	73.6	96.4	0.8
Noncationic tannage							
	Na <sub>2</sub> CO <sub>3</sub>	—	—	29.8	74.2	104.0	—
	Na <sub>2</sub> SO <sub>4</sub>	—	—	27.7	75.0	102.7	—
	Na <sub>2</sub> CO <sub>3</sub>	5.8	—	20.2	74.6	100.6	1.5
	Na <sub>2</sub> SO <sub>4</sub>	5.3	—	20.4	75.0	100.7	1.3
	Na <sub>2</sub> CO <sub>3</sub>	—	3.1	25.5	73.3	101.6	0.6
	Na <sub>2</sub> SO <sub>4</sub>	—	3.1	33.6	66.4	103.0	0.6

high as the takeup of acrylamide. In the first experiment, however, in which steerhide was treated with the monomers for 20 hr., the fixation in millimoles/g. collagen was the same for both, e.g., 1.4 after the cationic and 1.0 after the noncationic tannage. The longer reaction time benefited the takeup of acrylamide alone. Acrylonitrile, on the other hand, unstable as it is, shows no better fixation with time or concentration.

#### Influence of monomer upon aldehyde binding capacity.

Table I shows that after reacting with acrylonitrile or acrylamide first and subsequent tannage with formaldehyde, steerhide shrinks at 64° and 72° C. respectively. The fixation of H<sub>2</sub>CO by these hide pieces amounted to 22 and 35 % respectively of the quantities applied. Thus, in contrast to the  $T_s$ , formaldehyde fixation was not impaired at all by the presence of the monomer. In the case of acrylamide the affinity appeared to be even higher than that of normal cowhide, which, at the most, fixed 21% of the H<sub>2</sub>CO applied (4). In relation to the hide substance present in this modification nearly 1.1 millimoles/g. were fixed, i.e., a quantity of H<sub>2</sub>CO which, according to earlier findings (15), needed a much higher ratio of aldehyde to collagen.

#### DISCUSSION

The attachment of acrylonitrile and acrylamide to collagen causes a considerable reduction in  $T_s$  and curtails maximum chrome fixation. Although reaction conditions are fairly drastic, dry strength of the tanned leather is not affected. Therefore, we can assume that no degradation takes place under the conditions employed for the reaction of hide with the monomers, beyond the slight weakening of the fiber caused by the alkalis themselves. The present study does not provide the full answer to the question, Which active groups of the collagen molecule participate in the fixation of acrylonitrile or acrylamide? The most reactive groups, at the high pH required, are the ε-amino group of lysine and hydroxylysine residues; the guanidyl group, although considered very inert by comparison, can be partially diazotized (14b, 16) and at high pH values has also a remarkable affinity for formaldehyde (17). It is conceivable, however, that the guanidyl group does not participate to the same degree in the fixation of acrylonitrile or acrylamide. When alpha amino acids (18) or glycylglycine (19) are cyanoethylated, both hydrogens of the amino groups are substituted with ease. If this also is the case in collagen, a total of nearly 0.8 millimoles per gram collagen may combine in this manner, while only 0.3 millimoles at the most are left over for guanidyl residues. The tendency of forming ether links with hydroxyl groups appears to be negligible. A tricyanoethyl tyrosine was obtained in poor yield after 28 hr. of refluxing in a solution of caustic alkali (18b). At room temperature, in the presence of an insoluble protein, the conditions appear extremely unfavorable for this kind of substitution.

The nitrile group of a cyanoethylated material is very stable. It cannot be hydrolyzed without detriment to the long-chain molecules to which it is attached. Amide groups are more reactive. Carbamoylethylated collagen, therefore, might assume practical importance. It is able, for instance, to fix additional quantities of aldehyde. Its influence upon the fixation of vegetable or synthetic tannins has not been studied as yet.

Other advantages of acrylamide are that it is a crystalline solid, while acrylonitrile is a low boiling, highly toxic liquid. The solubility in water, of acrylamide as well as its stability at high pH values is very much greater than that of acrylonitrile. For operations on a larger scale acrylonitrile requires a completely closed system.

Acrylonitrile and acrylamide will probably not for long remain the only monomers which can be reacted with collagen under proper alkaline conditions. It also appears desirable to study the reactivity of collagen in other caustic solutions than those employed in this study. A third avenue of approach for the purpose of increasing the affinity of collagen for monomers would be the use of solvent systems which have shown promise in connection with a number of tannages.

#### SUMMARY

Strongly alkaline solutions in which the swelling of native hide is intentionally minimized offer satisfactory conditions for the cyanoethylation and carbamoylethylation of collagen. Under these conditions amounts as high as 1.4 millimoles per gram of collagen of either acrylonitrile or acrylamide have been introduced into the collagen molecule. The substitution with acrylamide appears to open possibilities of additional modifications; preliminary experiments point to an increased affinity for formaldehyde. Despite the fact that the maximum binding capacity for chrome and the thermal resistance are reduced, the strength properties are not affected. Finally, reasons are given why  $\epsilon$ -amino groups are believed to be primarily responsible for the reaction with the two monomers.

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