# 32. The Action of Formaldehyde on the Cystine Disulphide Linkages in Wool

## 1. The Subdivision of the Combined Cystine into Two Fractions differing in their Reactivity towards Formaldehyde

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Recent quantitative studies of the reaction of formaldehyde at ordinary temperatures with collagen and keratin (hair) have shown that in buffered solutions up to *p*H 8 containing 1 % HCHO, the terminal amino-groups of lysine combine with HCHO [Bowes & Pleass, 1939, 1, 2, 3; Highberger & Retzsch, 1939]. In solutions of higher *p*H the terminal guanidino-groups of the arginine side-chains also react. When more concentrated solutions of HCHO are used, Highberger & Salcedo [1940] found that the amount of HCHO with which collagen combined approached the limit of 2 mol. HCHO per free basic group.

Bowes & Pleass [1939, 2, 3], finding that deaminated hair combines with HCHO, have suggested that the cystine disulphide linkages of hair may be reduced by HCHO in alkaline solution and that the thiol groups produced may, like those of cysteine [Ratner & Clarke, 1937], condense with HCHO to give combined thiazolidine-4-carboxylic acid. Trotman *et al.* [1928] also noticed a difference in the firmness with which HCHO was held by collagen (skin) and by wool. They found that whereas all the HCHO could be removed rapidly in boiling water from skins, or from wool treated with cold solutions of HCHO, the HCHO fixed by wool at 75° was only removed very slowly. They also found that wool, after treatment with HCHO solutions at 75°, was more resistant to alkalis, and when boiled with dilute $H_2SO_4$ evolved HCHO until it was disintegrated.

By determinations of disulphide-S, we have found that below *p*H 7·0 at room temperatures HCHO does not react with the disulphide-S of wool. At 70° reaction takes place slowly and is apparently confined to about one-third of the total S. Evidence is put forward that only one S atom of each disulphide-S group that reacts is present in the treated wool as combined thiazolidine-4-carboxylic acid. Unlike ordinary wool [Elsworth & Phillips, 1938, 1; 1941], wool, after reaction with HCHO in this manner, does not give water-stable thiol and *S*-cysteinesulphonate groups with $NaHSO_3$.

### Experimental

*The inertness of wool disulphide-S to HCHO at 22° and p*H 6·9

Pieces of loosely woven, thin woollen fabric, prepared from 64–70's wool, were immersed for 48 hr. in 50 times their weight of 0·017 *M* $Na_2HPO_4$ at 22° containing 1 % HCHO; the *p*H was 6·9. After treatment, the wool was rinsed in water, dried at ordinary temperature and then conditioned.* The disulphide-S contents of the treated and untreated wools, determined by Shinohara's method [1935, 1], were respectively 2·81 and 2·80 %.† These results show clearly that at *p*H 6·9 and 22° HCHO does not react with the disulphide-S of wool, although Bowes & Pleass [1939, 1] found that hair exposed to an unbuffered 0·8 % solution of HCHO at *p*H 6·8 for 48 hr. fixed about 0·8 % of its weight of HCHO.

* All the wools described in this paper as conditioned had been exposed to a controlled atmosphere of 21° and 70 % R.H. until they attained constant weight.

† Unless otherwise stated, all analytical results are calculated on the weight of the anhydrous wool.

A further experiment showed that at higher *p*H some reaction with the disulphide-S occurs. Degreased, woven fabric (15 g.), which had been brought to equilibrium with water at *p*H 9, was immersed for 48 hr. in 1600 ml. of buffer of *p*H 9 containing 0·8 % HCHO. The buffer was 0·4 *M* in potassium dihydrogen phosphate, phenylacetic acid and boric acid and was brought to *p*H 9 by 0·2 *N* NaOH. The treated wool was rinsed thoroughly with water and then conditioned. By Bowes & Pleass's method [1939, 1] it was found to contain 1·17 % HCHO. The disulphide-S content was 2·75 % as compared with 2·94 % for the untreated wool. At *p*H 9·0 therefore the disulphide-S of wool reacts slowly with HCHO.

Separate experiments showed that HCHO did not interfere with Shinohara's method [1935, 1] for the determination of disulphide-S. Samples of wool were hydrolysed with 5*N* HCl and also with 5*N* HCl containing 0·8 % HCHO calculated on the weight of wool taken for hydrolysis. The determination of the disulphide-S in both hydrolysates gave the disulphide-S content of the wool as 2·80 %.

*The action of $NaHSO_3$ on formaldehyde-treated wools*

Wool, as loosely woven flannel, was treated with 50 times its weight of 1 % HCHO for 1 hr. at 17°. Another sample of the same wool was treated at 70°. The *p*H of the solutions during the treatment varied between 4·6 and 7·2. After treatment, the wools were rinsed in water and dried at room temperature. They were then immersed for 17 hr. in 40 times their weight of cold aqueous $NaHSO_3$ (*p*H 5·6) containing 3·0 % $SO_2$. The bisulphited wools were rinsed thoroughly in water, dried at room temperature and then conditioned. Their disulphide and thiol-S contents were determined by Shinohara's methods [1935, 1, 2, 3) and the total, 'free' and combined, $SO_2$ content by Elsworth & Phillips's methods [1938, 2]. Experiment showed that the addition of HCHO to a bisulphited wool before analysis by these methods did not affect the determination of the different classes of $SO_2$ which it contained.

Table 1. *The action of $NaHSO_3$ on wool after treatment with 1 % HCHO* (p*H 4·6–7·2*) *for 1 hr. at 18° and at 70°*

| Wool treated with HCHO: | Total $SO_2$ % | 'Free' $SO_2$ % | Combined $SO_2$ % | Thiol-S % | Disulphide-S % |
|---|---|---|---|---|---|
| At 18° | 1·18 | 0·50 | 0·68 | 0·42 | 1·98 |
| At 70° | 0·42 | 0·47 | 0·00 | 0·06 | 1·89 |

The disulphide-S content of the untreated wool was 2·80 %. From Table 1 it will be seen that this decreased to 1·89 %, when the wool was treated with HCHO at 70° and then the wool did not react with the $NaHSO_3$. On the other hand, after treatment with HCHO at 18°, the wool reacted with $NaHSO_3$ and its disulphide-S was lowered to 1·98 %. Treatment of the wool with either HCHO at 70° or with $NaHSO_3$ under the conditions of these experiments thus decreases the disulphide-S of the wool by approximately the same amount.

*The influence of the length of time of treatment of wool with (a) unbuffered, (b) buffered solutions of HCHO at 70° on the subsequent action of $NaHSO_3$ on the wool*

(*a*) *Unbuffered solutions.* The wool was immersed for the periods given in Table 2 in 50 times its weight of 1 % HCHO at 70°; the *p*H varied from 4·6 to 7·2 during the treatment. It was then rinsed in water, dried at room temperature and then conditioned. After the disulphide-S contents of the different samples had been determined, they were immersed for 17 hr. in 40 times their weight of aqueous $NaHSO_3$ (*p*H 5·6) at 70° containing 3·0 % $SO_2$. The bisulphited wools were rinsed thoroughly in water, and before being analysed by the methods previously mentioned, were dried at room temperature and conditioned. The results are given in Table 2.

(*b*) *Buffered solutions.* The above experiment was repeated using a 1 % solution of HCHO 0·02 *M* in $NaH_2PO_4$; the *p*H was 6·7–6·9. Samples of the formaldehyde-treated wools were immersed in $NaHSO_3$ under two sets of conditions: at the boil for 30 min. in 40 times their weight of aqueous $NaHSO_3$ at *p*H 5·8 containing 1 % $SO_2$; at room temperature for 24 hr. in 40 times their weight of aqueous $NaHSO_3$ at *p*H 5·5 containing 3·08 % $SO_2$. Analyses of the bisulphited wools are given in Table 2.

Table 2. *The decrease in the disulphide-S of wool on treatment with* (*a*) *unbuffered, and* (*b*) *buffered solutions of HCHO at 70° and its effect on the wool with* $NaHSO_3$

(*a*) Treatment with unbuffered solutions

| Time of treatment with HCHO hr. | Disulphide-S % | After treatment with $NaHSO_3$ at 70°: Disulphide-S % | Thiol-S in hydrolysate % | Combined $SO_2$ % |
|---|---|---|---|---|
| 0 | 2·80 | 1·94* | 0·83* | — |
| 0·17 | 2·81 | 2·11* | 0·35* | 0·32* |
| 1·0 | 2·67 | 2·26 | 0·09 | — |
| 5·0 | 2·51 | 2·19 | 0·19 | 0·19 |
| 48·0 | 2·17 | 2·24 | — | — |
| 72·0 | 2·00 | — | — | — |

(*b*) Treatment with buffered solutions

| Time of treatment with HCHO hr. | Disulphide-S % | After treatment with $NaHSO_3$: ½ hr. at 100° (1 % $SO_2$ : *p*H 5·8) Disulphide-S % | Thiol-S in hydrolysate % | 24 hr. at room *t*° (3·08 % $SO_2$ : *p*H 5·5) Disulphide-S % | Thiol-S in hydrolysate % |
|---|---|---|---|---|---|
| 0 | 2·80 | 1·94 | 0·66 | 1·94 | 0·83 |
| 3 | 2·65 | 2·02 | 0·49 | 2·06 | 0·18 |
| 18 | 2·49 | 2·17 | 0·34 | 1·92 | 0·16 |
| 48 | 2·12 | 2·10 | 0·28 | 2·02 | 0·03 |
| 96 | — | — | — | 1·91 | 0·02 |
| 120 | 1·98 | 2·03 | 0·21 | — | — |

* After treatment with $NaHSO_3$ at room temperature.

### *The influence of HCHO on the determination of thiol and disulphide-S by Shinohara's methods*

As mentioned previously, it was shown that wool could be hydrolysed in the presence of HCHO without affecting the accuracy of the determination of disulphide-S by Shinohara's method [1935, 1]. Further experiments were, however, necessary to test the accuracy of Shinohara's methods when applied to the determination of thiol-S [1935, 2, 3] and disulphide-S [1935, 1] in wools which had reacted with both HCHO and $NaHSO_3$, since the hydrolysates of such wools contain HCHO, cysteine and possibly thiazolidine-4-carboxylic acid. That some irregularities arise is shown by the analyses given in Tables 1 and 2. For example, only 0·42 % of thiol-S was found in the hydrolysate of the wool which had been treated with HCHO at 18° and then with $NaHSO_3$. Since each disulphide group which reacts with $NaHSO_3$ gives rise to two thiol groups when the wool is hydrolysed with HCl, the weight of thiol-S in the hydrolysate should equal the weight of combined $SO_2$. The combined $SO_2$ in this wool (Table 1) was 0·68 %, and hence it can be concluded that the thiol-S determination is incorrect, since 0·68 % thiol-S should have been found instead of only 0·42 %. Similarly, in Table 2, it will be seen that the sum of the thiol- and disulphide-S in the hydrolysates of the bisulphited wools is less than the amount of disulphide-S in the wools after treatment with HCHO.

Ratner & Clarke [1937] have shown that whereas cysteine in dilute HCl combines very slowly with HCHO, in solutions at *p*H 5 the reaction is very rapid. In Shinohara's method for the determination of thiol-S the hydrolysate is brought to *p*H 5, phosphotungstic acid is added immediately and the colour is allowed to develop for 20 min. Under these conditions, the length of time the cysteine and HCHO are free to react is short. If, on the other hand, some time is allowed to elapse before the phosphotungstic acid is added, then the cysteine and HCHO will combine and a less intense colour will be developed. For example, a bisulphited wool hydrolysate containing 1·0 % thiol-S (calculated on the weight of wool hydrolysed) was boiled with 1·0 % HCHO (also calculated on the weight of wool hydrolysed) for 1 hr. When phosphotungstic acid was added immediately after the hydrolysate had been buffered to *p*H 5, the coloration obtained corresponded to 0·95 % thiol-S, whereas when the acid was added after the buffered hydrolysate had stood for 1 hr., the coloration developed corresponded to only 0·56 % thiol-S.

In order to measure the effect of the HCHO concentration on the thiol-S determinations, samples of a bisulphited, but incompletely rinsed, wool were hydrolysed in the presence of increasing quantities of HCHO, and the thiol-S contents of the hydrolysates were determined after they had been buffered at *p*H 5 for 1 hr.

A further sample of this wool was hydrolysed and increasing quantities of HCHO were added to portions of the hydrolysate buffered at *p*H 5 and determinations of thiol-S were made after 1 hr. The thiol-S contents of these two sets of hydrolysates are given in Table 3. The analyses (Table 3) show that the amounts of thiol-S returned steadily decreased

Table 3. *The effect of HCHO on the thiol-S contents of the hydrolysates of bisulphited and rinsed wools*

| HCHO on weight of wool hydrolysed % | (*a*) HCHO added before hydrolysis | (*b*) HCHO added after hydrolysis |
|---|---|---|
| 0·0 | 1·37 | 0·89 |
| 0·25 | 1·01 | 0·61 |
| 0·50 | — | 0·48 |
| 1·0 | 0·42 | 0·34 |
| 3·0 | 0·00 | — |

as the concentration of HCHO increased, but the rate of decrease was the same whether the wool was hydrolysed in the presence of HCHO or whether the HCHO was added after hydrolysis. It is also evident that relatively large concentrations of HCHO must be present before the thiol-S in the wool hydrolysates of the nature we have examined would fail to give any coloration with phosphotungstic acid. Nevertheless, because of the presence of HCHO, the analyses of thiol-S in the formaldehyde-treated and then bisulphited wools (Table 2) are not of a high degree of accuracy. This lack of accuracy, however, does not invalidate the conclusions drawn, since these are based on the analyses of completely formaldehyde-treated wools that do not react with $NaHSO_3$ and did not therefore contain thiol-S.

A further possible loss of accuracy may be caused by the presence of thiazolidine-4-carboxylic acid groupings in formaldehyde-treated wools, since the thiazolidine-4-carboxylic acid liberated by hydrolysis may dissociate into cysteine and HCHO. Experiments were therefore made to test the stability of thiazolidine-4-carboxylic acid under the conditions used for the determination of thiol-S. The acid (150·6 mg.), M.P. 196–197°, prepared by Ratner & Clarke's method [1937], was heated under reflux for 4 hr. with 20 ml. 5*N* HCl in a flask fitted with a long air condenser. A thiol-S determination on the resulting solution showed that 5·5 mg. or 3·6 % of the acid had dissociated into cysteine and HCHO. When a solution of the acid (37 mg.) in 20 ml. of 5*N* HCl containing 1·0 g. of thiol-free gelatin was heated under reflux for 4 hr., 11·4 % of the acid dissociated. The

possible effect of the presence of combined thiazolidine-4-carboxylic acid in formaldehyde-treated wools on the thiol-S determinations is not likely therefore to have been large. For example, the disulphide-S content of a virgin Cape wool, purified as described by Elsworth & Phillips [1941], was reduced from 3·25 to 2·23 % by treatment with HCHO at 70°. Assuming that all the disulphide-S that disappeared became linked in thiazolidine-4-carboxylic acid groups, then the hydrolysate of 1·0 g. of this wool would contain 37 mg. of the acid. If, during hydrolysis, this acid behaved in the same manner as the acid hydrolysed in the presence of 1·0 g. of gelatin, then it would give rise to only 0·12 % thiol-S.

Since thiazolidine-4-carboxylic acid is decomposed by sulphite [Ratner & Clarke, 1937], it appeared more probable that this acid would interfere with the determinations of disulphide-S. Experiments were therefore made to obtain some measure of this interference. The results were of interest since they provided indirect evidence of the presence of thiazolidine-4-carboxylic acid in formaldehyde-treated wools. In these experiments advantage was taken of the slowness of the reaction between the acid and sulphite at *p*H 5. A virgin Cape wool which had been treated with HCHO at 70° was hydrolysed and the hydrolysate was buffered at *p*H 5 and then treated with sulphite as in Shinohara's method [1935, 1] for the determination of disulphide-S. The amount of cysteine the solution contained was then determined at intervals. Two 'synthetic' hydrolysates were also prepared and their disulphide-S contents determined at intervals in a similar manner. These hydrolysates were obtained by hydrolysing a quantity of untreated wool containing the same amount of disulphide-S as the formaldehyde-treated wool from which the first hydrolysate was prepared. Before hydrolysis was started, however, thiazolidine-4-carboxylic acid equivalent to half the difference between the total-S and disulphide-S of the formaldehyde-treated wool was added to one lot of the untreated wool, and to the other, thiazolidine-4-carboxylic acid equivalent to the difference between the total S and disulphide-S of the formaldehyde-treated wool. Further, since such wools would contain about 0·7 % HCHO linked to basic groupings, HCHO equivalent to 0·7 % of the weight of untreated wool hydrolysed was added to each solution before hydrolysis was started.

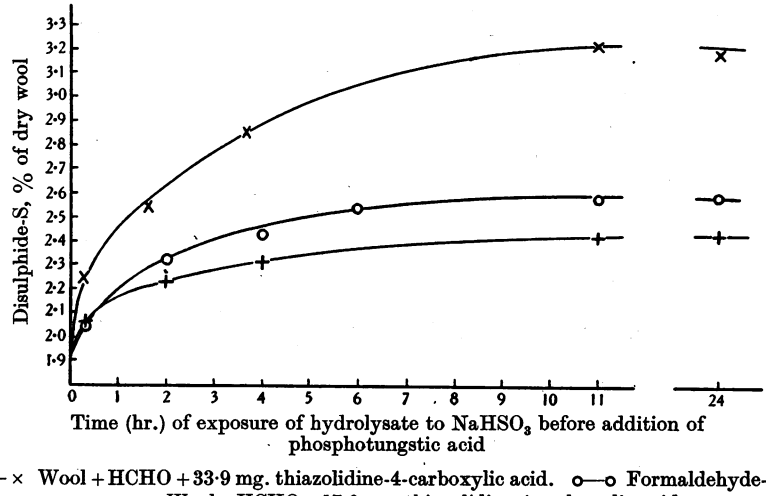


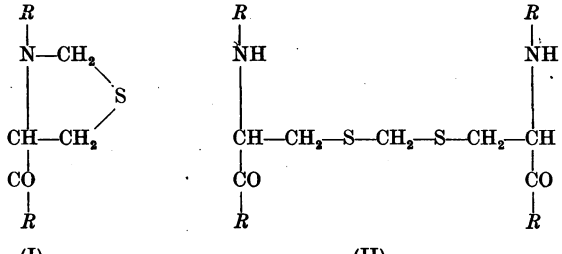

Fig. 1. ×—× Wool + HCHO + 33·9 mg. thiazolidine-4-carboxylic acid. o—o Formaldehyde-treated wool. +—+ Wool + HCHO + 17·0 mg. thiazolidine-4-carboxylic acid.

The results of the disulphide-S determinations on these three hydrolysates are plotted in Fig. 1 against the length of time the hydrolysate at *p*H 5 reacted with bisulphite before phosphotungstic acid was added and the determinations were made. If the increase in disulphide-S with time, which is shown by all the hydrolysates, can be taken to indicate dissociation of thiazolidine-4-carboxylic acid in the presence of sulphite, then it is evident

that the behaviour of the hydrolysate of the formaldehyde-treated wool is more closely imitated by the 'synthetic' hydrolysate containing the smaller quantity of the acid. In other words, the results suggest that only half the cystine-S which is lost when wool is treated with HCHO becomes part of a thiazolidine-4-carboxylic acid grouping. The analytical results support this suggestion closely. Thus the curve (Fig. 1) giving the disulphide-S content of the hydrolysate of the formaldehyde-treated wool, when extrapolated gives the disulphide-S at zero time as 1·92 %. The untreated wool contained 3·25 % disulphide-S. Hence 1·33 % disulphide-S has disappeared during the treatment of the wool with HCHO. Exactly half of this disulphide-S (0·66 %) is regained as thiol-S by exposing the hydrolysate to $NaHSO_3$ for 24 hr.

The curve relating to the hydrolysate of the formaldehyde-treated wool in Fig. 1 enables a small correction to be made to the determinations of disulphide-S recorded in Table 2. These determinations were made 20 min. after the addition of bisulphite to the buffered hydrolysates. From Fig. 1 it will be seen that at zero time the value for the percentage of disulphide-S is 0·10 less than the value at 20 min. The values for the disulphide-S contents of the completely formaldehyde-treated wools given in Table 2 are therefore greater by 0·10 than the true values.

Having obtained evidence of the presence of thiazolidine-4-carboxylic acid in formaldehyde-treated wool, it is interesting to note that the acid, as combined in wool, is relatively stable to $NaHSO_3$. This is shown by the failure to find thiol-S in formaldehyde-treated wools after they had been subjected to aqueous $NaHSO_3$ under various conditions. Even when such wools were immersed in boiling $NaHSO_3$ for 30 min. (Table 2) only small quantities of thiol-S were produced (0·21 %), being only slightly greater than the amounts produced (0·15 %) when the cold hydrolysate had been in contact with $NaHSO_3$ for 30 min. (Fig. 1). This is in agreement with the conclusion of Ratner & Clarke [1937] that acetylthiazolidine-4-carboxylic acid is much more stable to $NaHSO_3$ than is the unsubstituted acid. It thus provides some evidence that HCHO combines with wool as in (I).




(I) (II)

$R$ = polypeptide chain.

*The supercontraction of formaldehyde-treated hair*

Instead of condensing as in (I), the disulphide-S and HCHO may condense and produce djenkolic acid [Van Veen & Hyman, 1935] combined as in (II). The properties of this acid are similar to those of thiazolidine-4-carboxylic acid, although some doubt exists as to its stability towards HCl; Van Veen & Hyman [1935] reported that no cleavage occurred when it was boiled with varying concentrations of HCl, whereas Lillevik & Sandstrom [1941] state that it is decomposed into cysteine and HCHO to the extent of 85 % when boiled for 24 hr. with 20 % HCl.

The formation of combined djenkolic acid in this manner would produce stable cross-linkages between the polypeptide chains, and would explain the observed resistance of formaldehyde-treated wool to enzymic attack. For example, we have found that, although wool after treatment with HCHO at room temperature is rapidly disintegrated at 65°

by a solution of papain in $NaHSO_3$ [Middlebrook & Phillips, 1941], wool after treatment with HCHO at 70° is resistant to this enzyme solution.

The results of supercontraction experiments were, however, not in agreement with the presence of stable cross-linkages in formaldehyde-treated hair. Speakman [1936] found that when hair is boiled for 30 min. with 5 % $NaHSO_3$, it supercontracts 30 % in length when dried. Chemical treatments of hair, such as boiling with alkaline solutions [Speakman & Stoves, 1937], reduce the extent of the supercontraction, when the hair is subsequently boiled with $NaHSO_3$. It is considered [Speakman & Stoves, 1937] that this is due to the cross-linking of the polypeptide chains by stable covalent linkages, derived from the interaction of the disulphide-S with adjacent groupings. On this hypothesis, djenkolic acid cross-linkages of the type indicated in (II) would inhibit the supercontraction of keratin fibres. The supercontraction of degreased human hairs after treatment with HCHO at room temperature and at 70°, under the conditions given in Table 4, were therefore studied. The treated hairs were boiled in 5 % $NaHSO_3$ for 30 min., and the extent to which they contracted was measured before and after they had been air-dried.

Table 4. *The supercontraction of formaldehyde-treated hair when boiled in 5 % $NaHSO_3$ for 30 min.*

| | Supercontraction % | | | |
|---|---|---|---|---|
| | Untreated | 1 % HCHO (*p*H 5·6) at room temp. for 48 hr. | 1 % HCHO (*p*H 9·0) at room temp. for 48 hr. | 1 % HCHO (*p*H 5·6) at 70° for 48 hr. |
| Wet | 9·8 | 4·3 | 8·6 | 12·8 |
| Dry | 21·0 | 22·4 | 27·6 | 23·0 |

It is evident from these results that pre-treatment of hair either at room temperature or at 70° does not alter its power to supercontract when boiled with 5 % $NaHSO_3$. On the basis of the hypothesis that cross-linkage formation would prevent supercontraction, it appears that djenkolic acid is not produced (as in (II)) when hair reacts with HCHO. It might be suggested that djenkolic acid cross-linkages would be destroyed when the hair is boiled with $NaHSO_3$, but the analyses of formaldehyde-treated wool that has been boiled with $NaHSO_3$ give little support to this suggestion.

### *The influence of pH on the rate of reaction of wool with buffered solutions and with buffered solutions containing HCHO*

It is probable that HCHO does not react directly with the disulphide-S of wool, but as in the case of $NaHSO_3$ [Elsworth & Phillips, 1938, 1] reacts with the products of its hydrolysis:

$$—CH_2S.S.CH_2— \rightleftharpoons —CH_2SH + HOS.CH_2—$$

At room temperature, the rate of production of combined thiazolidine-4-carboxylic acid is slow, but at 70° and *p*H 5·6 it proceeds at a measurable rate. Even in the absence of HCHO, however, relatively large decreases in the disulphide-S of wool can be produced which become greater as the *p*H of the solution increases. Elsworth & Phillips [1941] showed, for example, that after wool had been boiled with a buffer at *p*H 8·5 for 30 min., it contained 1·06 % of non-disulphide-S* produced without loss of S. The loss of disulphide-S which occurs when wool is treated with solutions of HCHO at 70° is thus the result of two changes: the conversion of disulphide-S into non-disulphide-S, and the interaction of some of the thiol groups produced from the disulphide-S with HCHO. In order to compare the extents of these two changes, samples of Cape wool were immersed for increasing

* The term non-disulphide-S is used to describe S not returned either as disulphide-S or thiol-S by Shinohara's methods [1935, 1, 2, 3] or as methionine-S by Baernstein's method [1932].

periods in 50 times their weight of buffer solutions at *p*H 5·6 (0·1 *M* sodium acetate) and 8·5 (0·15 *M* borax with boric acid), and also in similar solutions containing 1·0 % HCHO. These solutions were kept at 70°, and the treated samples of wool, after rinsing, were analysed for disulphide-S. The results are plotted against the length of time of treatment in Fig. 2. It will be seen that in buffer solutions at *p*H 5·6, the disulphide-S content of the

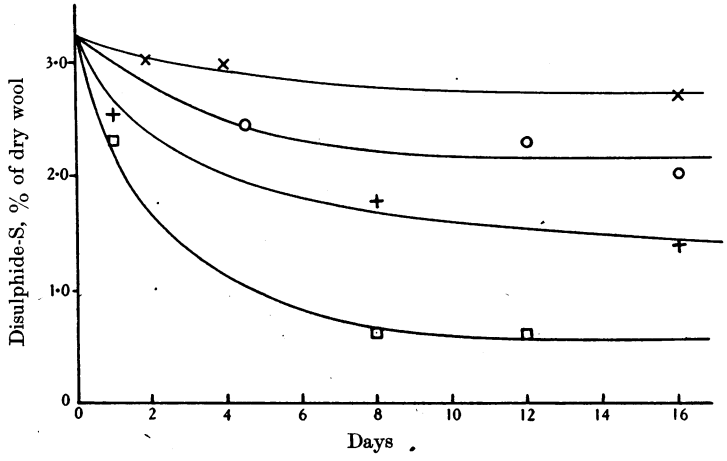



Fig. 2. ×—× Buffer *p*H 5·6. o—o Buffer *p*H 5·6 containing 1 % HCHO. +—+ Buffer *p*H 8·5. □—□ Buffer *p*H 8·5 containing 1 % HCHO.

wool changed slowly and reached a constant value of about 2·7 %. When the buffer contained HCHO, the disulphide-S fell more rapidly and tended to reach a constant value of 2·0 %. In buffer at *p*H 8·5, the disulphide-S of the wool fell much more rapidly and the rate of fall was maintained, until the value 1·4 % was reached. The presence of HCHO increased the rate of disappearance of disulphide-S which had fallen to 0·5 % in 16 days.

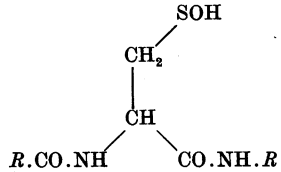
## Discussion

Elsworth & Phillips [1928, 1, 2; 1941] showed that approximately one-third of the disulphide-S of wool reacts with $NaHSO_3$ to give thiol and *S*-cysteinesulphonate groups which persist when the wool is rinsed with water. The present investigation has shown that these groups are not produced by the action of $NaHSO_3$ on wool which has been treated with HCHO at 70°. It thus appears that in solutions at *p*H 5·6 only about one-third of the disulphide-S of wool reacts with both $NaHSO_3$ and HCHO. Above *p*H 5·6, a larger proportion of the disulphide-S of wool is changed. Although this may be due partly to increased reaction with HCHO, it is mainly due to reaction of the disulphide-S with alkali. This leads to the production of non-disulphide-S with the simultaneous disappearance of an equivalent amount of disulphide-S.

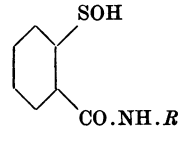
Should hydrolysis of the disulphide-S linkage precede condensation with HCHO, the formation of either combined djenkolic or thiazolidine-4-carboxylic acid involves assumptions as to the fate of the sulphenic acid group (III). If the HCHO condenses with the thiol group, the —S.$CH_2$.OH group produced can only yield combined djenkolic acid, when the sulphenic acid group has been reduced to a thiol group. On the other hand, if the thiol group, produced by hydrolysis of the disulphide-S group, condenses with HCHO to give combined thiazolidine-4-carboxylic acid, then either the sulphenic acid group remains free or reacts with one or more adjacent groups.

There is no evidence that the sulphenic acid group is reduced under the conditions of our experiments. Further, it is unlikely that the sulphenic acid group remains free and un-

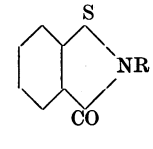
changed, since it would then persist in the acid hydrolysates of formaldehyde-treated wool. Here it would be detected by its reducing action on the phosphotungstic acid reagent [Clarke, 1932]. No such reducing action was observed. On the assumption therefore that one S atom only of the disulphide-S group condenses with HCHO, the fate of the S atom of the sulphenic acid group remains obscure, the only likely suggestion that might be made at present being that this group condenses with the adjacent imino-group of the polypeptide chain in the same manner as the sulphenic acids derived from substituted amides (IV) condense to give ketobenz*iso*thiazoles (V) [McClelland & Warren, 1930].




(III)




(IV)




(V)

$R$ = polypeptide chain.

## Summary

1. At 70°, but not at room temperature, wool reacts with HCHO in solutions at *p*H 5·6, and combined thiazolidine-4-carboxylic acid equivalent to half the disulphide-S that disappears is probably produced. Only about one-third of the total disulphide-S of wool reacts in this manner.

2. Wool that has reacted with HCHO at 70° does not react with $NaHSO_3$ to give thiol and *S*-cysteinesulphonate groups that are stable to water.

3. It is probable that the fraction of the disulphide-S of wool which reacts with $NaHSO_3$ to give water-stable thiol and *S*-cysteinesulphonate groups is also the fraction of the disulphide-S that reacts with HCHO at *p*H 5·6 and 70°.

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